"Hematological and Imaging Parameters in prediction of adverse outcomes in Adult Sickle Cell Anaemia"

Thesis submitted to the faculty of
ALL INDIA INSTITUTE OF MEDICAL SCIENCES, BHOPAL

for the award of the degree of MD in the subject of General Medicine

by

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ACKNOWLEDGEMENT

I would like to express my sincere gratitude to all those who have contributed to the successful completion of this thesis on "Haematological and Imaging Parameters in Prediction of Long-Term Adverse Events in Adult Sickle Cell Anaemia."

First and foremost, I am deeply indebted to my guide, Dr. Vaibhav Ingle, for his exceptional guidance, invaluable insights, and constant support throughout the course of this work. His mentorship has been the cornerstone of this thesis.

I also extend my heartfelt thanks to my co-guide, Dr. Rajnish Joshi, Head of Department, for his invaluable input, wisdom, and support in providing the necessary resources for this research.

My profound appreciation goes to Dr. E. Jayashankar, Additional Professor, Department of Laboratory Medicine & Pathology, for his expert advice and support, which were crucial in shaping the direction of this study.

I am equally grateful to Dr. Sachin Bansal, Assistant Professor, Medical Oncology and Hematology, for his expert guidance and support in the hematological aspects of this thesis. I would also like to thank Dr. Jitendra Sharma, Assistant Professor, Department of Radiodiagnosis, for his assistance in the imaging components of the study, and Dr. Suruchi Jain, Assistant Professor, Department of Nuclear Medicine, for her valuable contributions to the nuclear imaging aspects.

I am immensely thankful to my teachers, Dr. V.N. Mishra, Dr. Seema, Dr. Sagar, Dr. Abhishek, Dr. Yasmee, Dr. Mahadev Meena, Dr. Akash, and Dr. Sukumar, for their guidance and encouragement throughout my academic journey.

I would like to express my deepest gratitude to my parents, Dr. Arun Madharia and Dr. Sandhya Madharia, for their endless love, support, and inspiration, which have been my greatest motivation.

My heartfelt thanks also go to my siblings, Dr. Divya Dausage, Dr. Ankit Madharia, Dr. Chirag Dausage, Dr. Shilpa Madharia, and Dr. Astha Madharia, for their constant encouragement and support. Also to my constant, Dr Shreya.

To my friends, Dr. Tejaswita Chourey, Dr. Shaurya Tewari, and Dr. Sanskriti Kalura, I am grateful for their friendship and unwavering support during this journey.

I am also blessed to have the love and joy brought into my life by my wonderful nieces and nephews, Avani, Krishav, Aarav, and Raghav. Their smiles and laughter have been a source of immense happiness and motivation.

Lastly, I would like to thank the senior residents, and all my seniors and juniors, for their support, collaboration, and valuable insights throughout the course of this work.

Thank you all for your invaluable contributions, without which this thesis would not have been possible.

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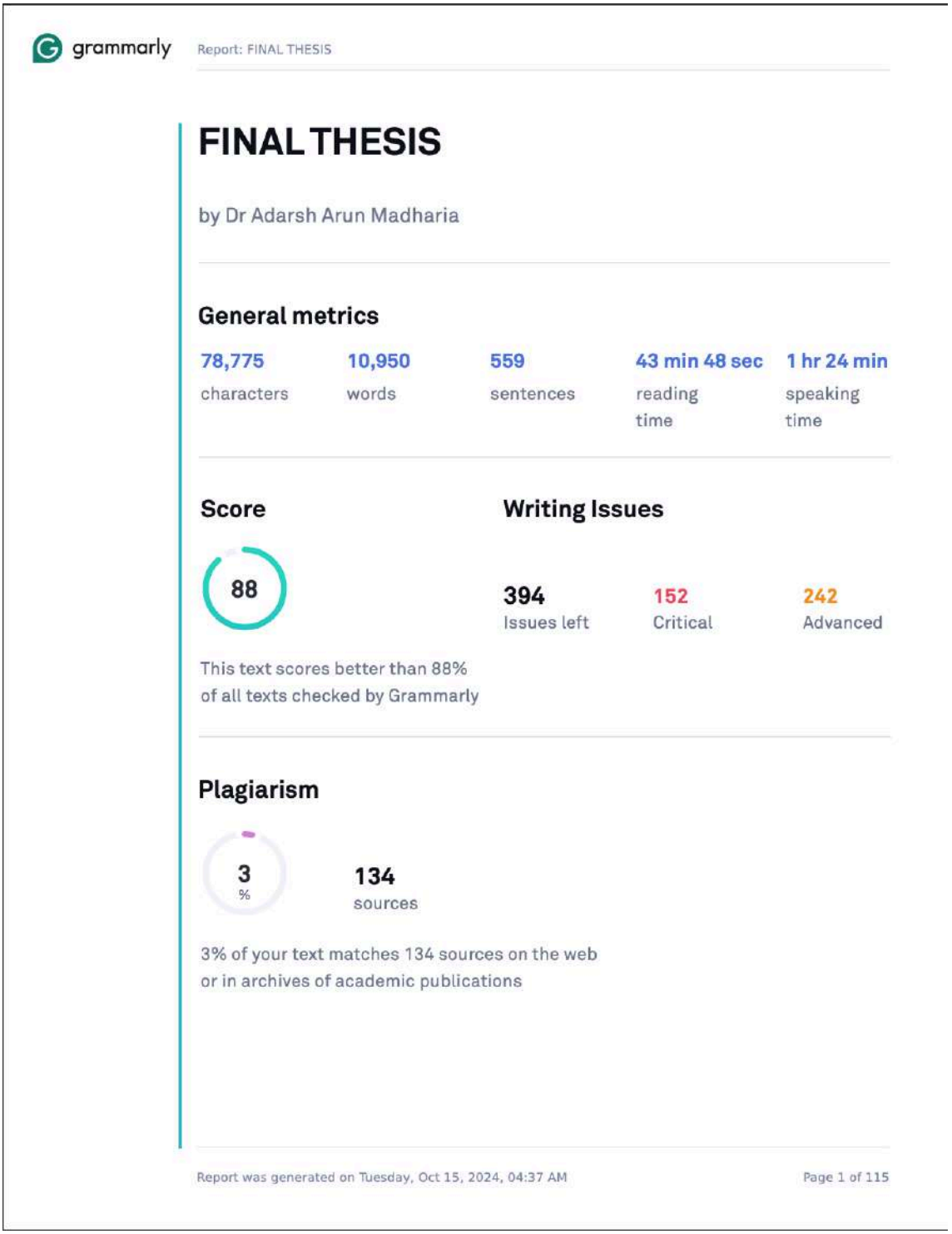
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Abbreviations :

ACS - Acute Chest Syndrome
ADMA - Asymmetric Dimethylarginine
AIIMS - All India Institute of Medical Sciences
APL - Above Poverty Line
AVN - Avascular Necrosis
BPL - Below Poverty Line
BNP - Brain Natriuretic Peptide
CCF - Congestive Cardiac Failure
CKD - Chronic Kidney Disease
DVT - Deep Vein Thrombosis
ET1 - Endothelin-1
eGFR - Estimated Glomerular Filtration Rate
FA - Folic Acid
FVC - Forced Vital Capacity
Hb - Hemoglobin
HbA - Hemoglobin A
HbA0 - Hemoglobin A0
HbA2 - Hemoglobin A2
HbAS - Sickle Cell Trait (HbA and HbS)
HbC - Hemoglobin C
HbD - Hemoglobin D
HbE - Hemoglobin E
HbF - Fetal Hemoglobin
HbHPLC - Hemoglobin High-Performance Liquid Chromatography
HbO - Hemoglobin O
HbS - Sickle Hemoglobin
HbS/HPFH - Sickle Hemoglobin with Hereditary Persistence of Fetal Hemoglobin
HbSC - Hemoglobin S and C Disease
HbSB+ - Hemoglobin S with Beta Plus Thalassemia
HbSB0 - Hemoglobin S with Beta Zero Thalassemia
HbSE - Hemoglobin S with Hemoglobin E Disease
HbSS - Sickle Cell Anemia (Homozygous Sickle Hemoglobin)
HDL - High-Density Lipoprotein
HPFH - Hereditary Persistence of Fetal Hemoglobin
HR - Hazard Ratio
ICAM-1 - Intercellular Adhesion Molecule 1
ICMR - Indian Council of Medical Research
IHEC - Institutional Human Ethics Committee
IQR - Interquartile Range
LDH - Lactate Dehydrogenase
LV - Left Ventricle
LVEF - Left Ventricular Ejection Fraction

MACRO - Macroalbuminuria
MICRO - Microalbuminuria
MP - Madhya Pradesh
MRI - Magnetic Resonance Imaging
MCHC - Mean Corpuscular Hemoglobin Concentration
MPV - Mean Platelet Volume
NCD - Non-Communicable Disease
NGAL - Neutrophil Gelatinase-Associated Lipocalin
NO - Nitric Oxide
NT-proBNP - N-terminal pro-Brain Natriuretic Peptide
OPD - Outpatient Department
PFT - Pulmonary Function Test
PLT - Platelets
PRBC - Packed Red Blood Cells
RBC - Red Blood Cell
RNR - Ribonucleotide Reductase
ROS - Reactive Oxygen Species
RV - Right Ventricle
SCN - Sickle Cell Nephropathy
SCT - Sickle Cell Trait
SCD - Sickle Cell Disease
SS - Homozygous Sickle Cell (HbSS)
suPAR - Soluble Urokinase-type Plasminogen Activator Receptor
SYS - Systolic Dysfunction
TAPSE - Tricuspid Annular Plane Systolic Excursion
TLC - Total Leukocyte Count
T2-BLACKOUT - T2 Blackout Effect
VCAM-1 - Vascular Cell Adhesion Molecule 1
VOC - Vaso-Occlusive Crisis
VTE - Venous Thromboembolism
WBC - White Blood Cell

Introduction

Sickle Cell Disease (SCD) is a genetic disorder first described in 1910 by Dr. James Herrick, who documented a patient with abnormal, "sickle-shaped" red blood cells (RBCs).[1] SCD is caused by a mutation in the β-globin gene, producing abnormal hemoglobin (HbS). Under conditions of hypoxia, this defective hemoglobin polymerizes, causing RBCs to become rigid, assume a sickle shape, and ultimately contribute to vaso-occlusion and hemolysis. As a result, patients experience painful crises and multi-organ complications, ranging from bone and joint issues to renal and cardiovascular dysfunction.

Globally, SCD affects an estimated 20 million individuals, with over 300,000 infants born each year with the severe form of sickle cell anemia (SCA).[2] The disease is most prevalent in regions where malaria has been historically endemic, including sub-Saharan Africa, India, and parts of the Mediterranean and Middle East.[3] As the global burden of the disease becomes clearer, SCD is increasingly being recognized as a major public health issue, particularly in low- and middle-income countries, where healthcare resources and access to comprehensive care are limited.[4]

India has one of the largest populations of individuals with SCD outside of Africa. The disease is particularly common in tribal populations of Central India, where both the trait and disease have been noted to have a prevalence of up to 22%.[5] States like Madhya Pradesh, Chhattisgarh, Maharashtra, Odisha, and Gujarat are home to large populations affected by SCD, with nearly 20 million people carrying the sickle cell trait.[6]

A 2018 report by the Indian Council of Medical Research (ICMR) estimated that 1.4 million people in India live with SCD .[6] Despite this, SCD remains an under-recognized and under-researched health issue in India, primarily due to the socio-economic status of affected populations. Many patients come from marginalized tribal communities, which face limited access to healthcare, delayed diagnosis, and inadequate follow-up care.[7]

SCD's status as an understudied disease stems from a complex interplay of medical, social, and economic factors. In India, healthcare priorities have traditionally been focused on infectious diseases like tuberculosis and malaria, with non-communicable diseases (NCDs) receiving relatively little attention.[7] This neglect has resulted in fewer resources dedicated to research, management, and prevention strategies for SCD.[8] Moreover, the populations most affected by SCD often have limited political or economic influence, which further hampers the disease's visibility on national health agendas. Additionally, misconceptions about the disease and its heritability have contributed to its stigmatization, particularly in rural and tribal communities.[9]

However, SCD has transformed from a fatal childhood illness to a chronic condition affecting adults, thanks to advancements in public health measures and treatments over the past 40 years.[10] This shift has led to increased survival rates, but also new challenges as patients transition from pediatric to adult care.[11] Adult SCD patients face accumulating comorbidities and complications, requiring adult healthcare providers to become more familiar with the disease and its long-term management.[12] It is known that the clinical course of SCD in adults is highly variable, yet the predictors of this variability, particularly in Indian populations, remain poorly understood.

Several studies have indicated the role of hematological parameters in predicting the frequency of VOCs and the severity of complications in SCD. For instance, a study by Platt et al. in 1994 found that hemoglobin levels, white blood cell (WBC) counts, and fetal hemoglobin (HbF) levels were significant predictors of mortality in SCD. Patients with lower HbF levels and higher WBC counts had a greater risk of VOCs, acute chest syndrome, and early mortality.[13] However, the need for collaborative research between high-income countries and low-resource settings has been emphasized to improve clinical care and develop national guidelines for SCD management in India.[14]

Similarly, studies have suggested that hemoglobin variants, particularly the presence of HbS and HbF, can modulate disease severity. For instance, Ballas et al. (2010) demonstrated that higher levels of HbF are associated with fewer pain crises and better long-term outcomes, as HbF inhibits HbS polymerization, reducing the severity of hemolysis and vaso-occlusion[15]. However, the levels of HbF can vary widely between populations, and its correlation with clinical outcomes in Indian SCD patients remains under-researched.

The current study seeks to fill this gap by focusing on adult SCD patients in a tertiary care setting in Central India. By correlating hematological parameters (e.g., hemoglobin levels, hemoglobin variants) with imaging findings and the frequency of acute crises and chronic complications, this study aims to provide insights into the predictors of disease severity in this population. Furthermore, the study will explore the utility of imaging techniques in assessing chronic complications like AVN and other organ damage, which are common yet underreported in Indian SCD populations.

By identifying correlations between hematological and imaging parameters with clinical outcomes, this study aims to improve the early detection of complications, guide treatment decisions, and contribute to the development of personalized management strategies for adult SCD patients in India. Understanding these correlations will also help to bridge the knowledge gap between pediatric and adult SCD care, ensuring that more patients not only survive into adulthood but also enjoy improved quality of life and reduced morbidity.

Research Question: Can hematological (hemoglobin levels, hemoglobin variants) and imaging (X ray, M.R.I., Bone Scan) parameters in adult Sickle Cell Anemia be predictive of the frequency of acute crises and the development of chronic complications?

Review of Literature

Sickle cell disease (SCD) is a hereditary blood disorder characterized by the production of abnormal hemoglobin, which leads to the distortion of red blood cells (RBCs) into a sickle or crescent shape.[16] These misshapen cells can cause blockages in small blood vessels, leading to reduced blood flow and oxygen delivery to various parts of the body. This results in a range of symptoms, including severe pain episodes, chronic anemia, frequent infections, and potential organ damage.[16] Sickle cell disease primarily affects individuals of African, Mediterranean, Middle Eastern, and Indian ancestry, though it can occur in other populations as well.[17] Despite being a well-recognized condition, SCD poses ongoing challenges in terms of management and treatment, and significant research is underway to improve therapies and enhance the quality of life for those affected.

Epidemiology

SCD has long been an overlooked health issue in India, with limited research available. Research on SCD is predominantly confined to biomedical fields such as hematology, pathology, and genetics, and lacks a comprehensive health systems perspective.[18,19] According to the World Health Organization, a health system encompasses all organizations, people, and actions focused on promoting, restoring, or maintaining health, with the aim of improving health equity in a responsive and efficient manner.[20] Currently, there is no national or state-led initiative providing a health systems approach to SCD, although the Ministry of Health and Family Welfare has proposed a draft policy.[21] In contrast, some non-governmental organizations have made significant efforts to address SCD in remote tribal areas, but these initiatives often lack coordination with public health services.[17]

India has the second most significant global health burden of SCA worldwide, after Africa, with a notably high prevalence in certain regions, particularly among tribal populations in central and eastern India.[22] Within these tribal communities, the prevalence of sickle cell traits is strikingly high, reaching levels between 40% and 55% in some areas.[8] This is especially evident in states such as Madhya Pradesh, Gujarat, Odisha, and Jharkhand, where high carrier rates are prevalent. For instance, within specific tribal groups in Madhya Pradesh, the prevalence of the sickle cell trait can

exceed 30%.[23] In contrast, the prevalence of SCD in the broader Indian population is relatively lower, with estimates ranging from approximately 0.1% to 0.2%, though these figures can vary depending on regional studies and demographic specifics.[24] Early cross-sectional surveys have documented significant variability in SCD prevalence among different communities, with rates ranging from as low as 1% to as high as 55% in certain tribal regions.[8] These surveys underscore the considerable variation in SCD prevalence both within and between different communities, reflecting the diverse epidemiological landscape of the disease across India.

Pathophysiology

SCD includes various genetic mutations in the β-globin gene that lead to a similar set of clinical symptoms. The most common form, sickle cell anemia, accounts for the majority of SCD cases. This condition is caused by homozygosity for the βS allele on chromosome 11p15.5, resulting from a single nucleotide substitution (GTG to GAG) in the sixth codon of the β-globin gene.[25] This mutation replaces a hydrophilic glutamic acid with a hydrophobic valine, producing hemoglobin S (HbS) in those affected.[26] Individuals with homozygosity for the βS allele develop sickle cell anemia (HbSS), while other forms of SCD arise from the combination of βS with other mutations such as βC, βD, βO, βE, or β-thalassemia, each leading to different types of sickling disorders.[27,28]

Research has highlighted three primary pathobiological processes driving SCD: HbS polymerization, vaso-occlusion, and hemolysis-induced endothelial dysfunction.[29] More recently, sterile inflammation has also been identified as a significant contributing factor.[30]

Figure 1 : A detailed illustration of the genetic variations in sickle cell disease and their effects on hemoglobin structure and function.

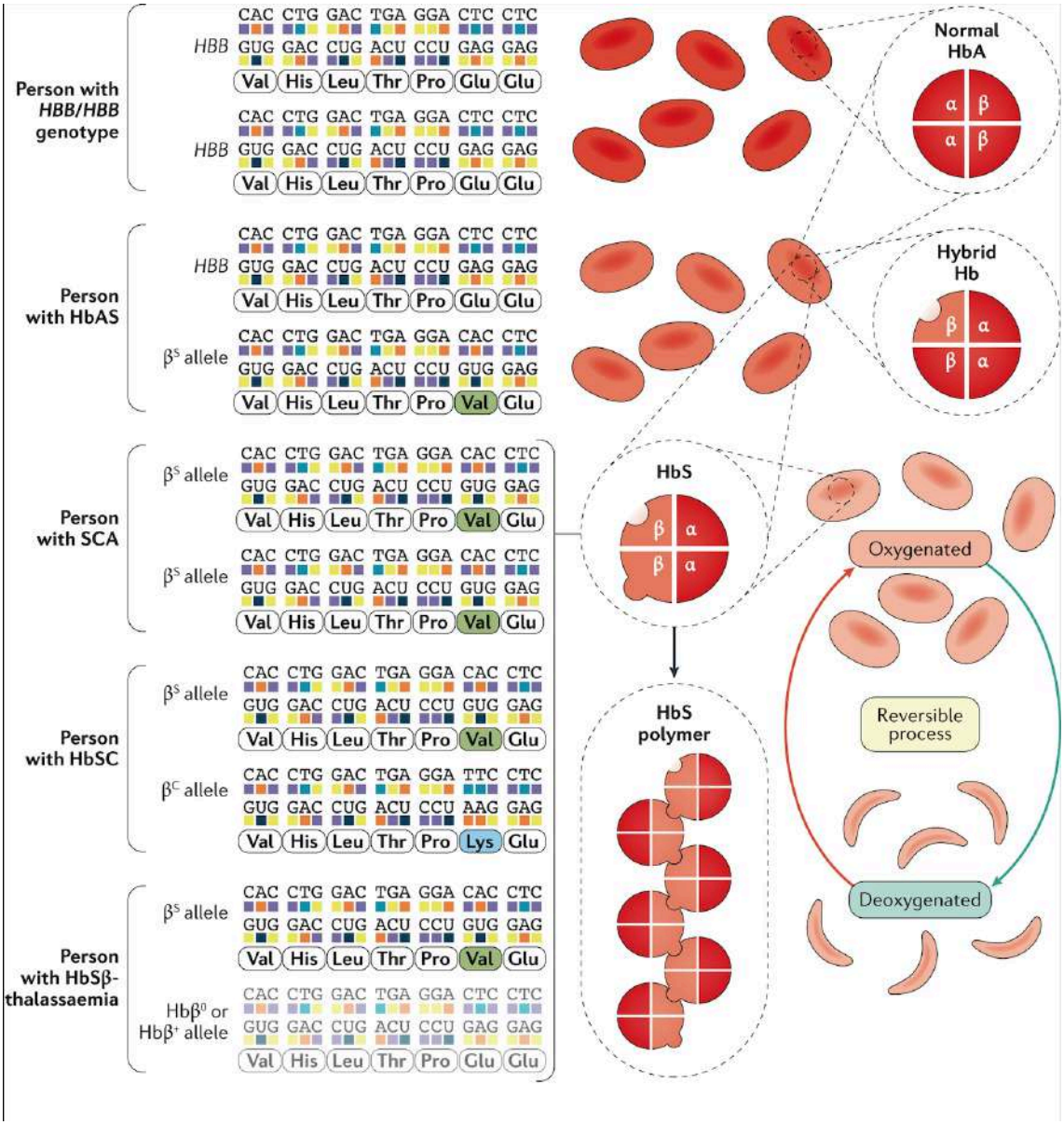


Image source : Kato GJ, Piel FB, Reid CD, et al. Sickle cell disease. Nat Rev Dis Primers. 2018;4:18010. Figure 1.

In normal physiology, hemoglobin A (HbA) is the predominant form of hemoglobin in adults and is composed of two α-globin chains and two β-globin chains. This structure allows HbA to efficiently bind and transport oxygen throughout the body, remaining

soluble in both its oxygenated and deoxygenated states, thereby facilitating smooth and effective oxygen delivery to tissues. In individuals with SCD, however, the mutation results in the production of hemoglobin S (HbS). Unlike HbA, HbS molecules contain valine residues that promote aggregation and polymerization under low-oxygen conditions. These hydrophobic interactions cause HbS molecules to stick together, forming long, rigid polymers.[31] This polymerization leads to the distortion of RBCs into a characteristic sickle shape, significantly impairing their flexibility and ability to navigate through microvasculature, thereby initiating a cascade of pathological events that are central to the disease's complications.[31]

Sickle cell trait (SCT) is characterized by the heterozygous presence of one HbA and one HbS. It has traditionally been viewed as benign due to the absence of severe symptoms associated with SCD. SCT provides an evolutionary protection against severe Plasmodium falciparum malaria, reducing the risk of complications like cerebral malaria, although the exact mechanisms are not fully understood. Additionally, while individuals with SCT usually have normal RBC shape, extreme conditions such as hypoxia, high altitude, dehydration, and intense physical exertion can cause transient sickling, potentially leading to acute complications.[32]

SCT is associated with several clinical complications. Renal complications are common, including hematuria and reduced urinary concentrating ability, with SCT carriers facing a 1.57-fold increased risk of chronic kidney disease (CKD). Additionally, there is a strong association between SCT and renal medullary carcinoma.[33,34] Tsaras et al., and Naik Haywood et al., have shown that individuals with SCT have an elevated risk of venous thromboembolism (VTE), particularly pulmonary embolism (PE), though deep vein thrombosis (DVT) risk does not seem to be significantly higher. SCT carriers are approximately twice as likely to experience PE, especially under conditions like dehydration or immobility.[32,33]

Hulsizer et al. indicates that SCT may increase the risk of ischemic stroke, particularly under hypoxic stress, although the exact mechanisms are still being studied, and the

connection to overall cardiovascular disease risk remains uncertain.[35] Pregnancy-related complications in SCT carriers may include preeclampsia, fetal loss, and low birth weight, with some evidence suggesting a higher incidence of asymptomatic bacteriuria among pregnant women with SCT, although these findings are not consistently confirmed.[36]

Hemoglobin SC disease (HbSC) occurs when an individual inherits one HbS gene and one hemoglobin C gene (HbC), resulting in a generally milder clinical presentation than SCD. Vaso-occlusive events, though less frequent than in HbSS SCD, can still lead to significant pain crises. Additionally, splenomegaly may develop over time, potentially leading to functional asplenia, which compromises the spleen's filtering and immunologic abilities. Da Guarda et al showed that while HbSC disease shares some complications with HbSS, such as stroke, acute chest syndrome, VTE and an increased risk of infections, these occur less frequently and are often less severe.[37] Although Dass et al, had shown that HbSC is extremely rare in the Indian setting.[38]

HbS can be combined with various β-thalassemia mutations, resulting in a range of clinical presentations from nearly asymptomatic to severe forms indistinguishable from sickle cell anemia. While there is no consensus on classifying HbS/β-thalassemia, it is generally categorized into HbS/β^0-thal, which completely lacks HbA production, and HbS/β^+-thal, which has some, but deficient amount of HbA production. HbS/β^0-thal often mimics sickle cell anemia, leading to microcytosis, hypochromia, and sometimes elevated HbF, improving circulatory function and reducing hemolysis without decreasing vaso-occlusive events due to increased blood viscosity. Differentiating HbS/β^0-thal from sickle cell anemia with α-thalassemia is challenging, as standard hematologic and electrophoretic tests do not provide clear distinctions; thus, family studies and DNA analysis are necessary for accurate diagnosis.[39,40].

Hemoglobin SE disease results from inheriting one HbS gene and one hemoglobin E gene (HbE), typically leading to mild to moderate anemia. Usually, the composition of hemoglobin is in the range of 60-65% HbS and 30-35% HbE. While often considered

milder than sickle cell anemia, HbSE can still present significant complications, including vaso-occlusive crises, acute chest syndrome, and hemolytic anemia due to unstable hemoglobin. Other potential issues include splenomegaly, stroke, bone infarction, thromboembolic events, gallstones, and an increased risk of infections due to impaired splenic function.[41] HbE is mainly restricted in tribals of North-East, West Bengal, Odisha and those in Andaman and Nicobar islands.[42]

Hemoglobin S/Hereditary Persistence of Fetal Hemoglobin (HbS/HPFH) is a sickle cell variant where there is one HbS gene, and one gene for continual production of HbF (fetal hemoglobin). This causes the levels of HbF to naturally stay higher, inhibiting the HbS polymerisations and thus exerting a protective effect against the injurious effects brought about by sickling. In patients with HbS/HPFH, the hemoglobin levels tend to remain closer to normal, with most individuals experiencing a mild or asymptomatic anemia, and very few episodes of vaso-occlusion. Overall, these individuals remain asymptomatic throughout life, without complications such as organ damage, chronic pain, or delays in growth seen in other sickle cell disorders. Serious complications like acute chest syndrome, stroke, and chronic hemolysis are relatively uncommon.[43]

Hemoglobin S/Lepore Thalassemia is a rare condition resulting from one HbS gene and one hybrid beta-thalassemia gene (Hb Lepore), leading to moderate anemia and mixed clinical features of both sickle cell disease and thalassemia. Patients typically experience moderate, microcytic anemia with infrequent vaso-occlusive crises, which can occur during stress but are generally milder than in HbSS. Complications include chronic hemolysis, leading to jaundice and gallstone formation, ineffective erythropoiesis, and potential iron overload, particularly in those requiring blood transfusions. Prognosis is better than HbSS disease, with much lower risks of potential life threatening complications.[44]

The cyclical sickling and unsickling of RBCs cause cumulative membrane damage, leading to calcium influx into the cells.[45] Elevated calcium levels activate the Gardos channel, resulting in potassium loss and cellular dehydration, further exacerbating HbS

polymerisation.[46] This dehydration reduces RBC deformability and increases the mean corpuscular hemoglobin concentration (MCHC).[47] An increased MCHC further promotes HbS polymerization, exacerbating dehydration in a vicious cycle. This cycle causes more membrane damage, calcium influx, and additional dehydration. Furthermore, sickling and dehydration disrupt the normal phospholipid asymmetry of RBC membranes, increasing their adhesiveness.[48]

The sickled cells, which are less flexible than normal biconcave discs, struggle to navigate through the microvasculature, leading to two major issues: vaso occlusion and hemolysis.[29,49] The sickled cells adhere more strongly to endothelial cells and clump together with platelets and leukocytes, causing blockages in the microvasculature. This results in ischemia, pain crises, and potential damage to multiple organs.[50] Additionally, the sickled cells activate the endothelium to produce adhesion molecules such as VCAM-1 and ICAM-1, which further promote the trapping of sickled cells and leukocytes, exacerbating vessel blockages and creating an inflammatory environment.[51] The rigidity of sickled cells also makes them prone to premature destruction, or hemolysis, either within blood vessels or in the spleen. This hemolysis contributes to chronic anemia and releases free hemoglobin into the bloodstream, which scavenges nitric oxide (NO), a key vasodilator.[52,53] Reactive oxygen species, including superoxide, are elevated and rapidly react with NO to form peroxynitrite, thereby reducing NO availability.[54] Additionally, elevated levels of xanthine oxidase further contribute to the production of superoxide. Hemolysis also releases cell-free hemoglobin, which directly releases arginase, an enzyme that converts arginine (a precursor for NO production) into urea and ornithine, thus depleting NO synthesis. This combination of increased NO scavenging and reduced arginine availability exacerbates the low NO levels seen in sickle cell anemia, contributing to the disease's vascular complications.[55]. The depletion of NO impairs blood vessel dilation, leading to complications such as pulmonary hypertension[56] and worsening vaso-occlusive pain.[57]

Imaging Parameters and Characteristics in Sickle Cell Disease

- *Cardiopulmonary Imaging*

Echocardiography [58]

Left Ventricular (LV) Dysfunction [59–62]

Left ventricular (LV) dysfunction in SCD arises from the chronic high-output state due to anemia, leading to compensatory hyperdynamic circulation. Over time, this can manifest as both systolic and diastolic dysfunction. Systolic dysfunction occurs due to chronic volume overload, causing myocyte hypertrophy and reduced contractility. Diastolic dysfunction results from impaired relaxation and changes in ventricular compliance, contributing to elevated filling pressures.

Echocardiographic evaluation of LV systolic dysfunction involves several advanced measurements. For systolic function, key findings include reduced left ventricular ejection fraction (LVEF), with normal values ranging from 55-70% and values below 50% indicating dysfunction. Fractional shortening (FS) and global longitudinal strain (GLS) are also critical metrics; normal FS values are 25-45%, while GLS (ideally between -18% to -22%) can detect latent dysfunction even when LVEF appears normal.

For diastolic dysfunction, impaired relaxation and increased filling pressures are assessed through parameters such as the E/A ratio, with a normal range of 1-2. An E/A ratio less than 1 indicates impaired relaxation, while greater than 2 suggests restrictive filling. The E/e' ratio, with values exceeding 14 indicating increased left atrial pressures, is another important marker of diastolic dysfunction. Lastly, the left atrial volume index (LAVI) provides insights into chronic diastolic dysfunction, with normal values being less than 34 mL/m². Elevated LAVI suggests increased left atrial pressures due to sustained diastolic dysfunction.

-Musculoskeletal Imaging

X-Rays

X-ray imaging in SCD reveals various musculoskeletal complications that reflect the disease's impact on the bones and joints. One prominent finding is sickle cell osteomyelitis, which presents on X-rays with bone destruction and irregularities. Affected bones may show radiolucent areas indicating bone loss or abscess formation, along with periosteal reaction characterized by new bone formation or thickening of the periosteum adjacent to the infection site.[63]

Bone infarcts and avascular necrosis (AVN), particularly in the femoral head, often appear as subchondral sclerosis with associated cystic lesions. A crescent sign, seen as a subcortical radiolucent line at the weight-bearing surface, is an early indicator of AVN. Over time, these infarcts lead to collapse of the bone structure, visualized as deformities or flattening of the femoral head, with irregular bone contours and joint space narrowing.[64]

Chronic anemia in SCD leads to increased hematopoiesis, causing bone marrow hyperplasia. X-rays show medullary expansion with increased radiopacity due to the replacement of normal fatty marrow with hyperplastic hematopoietic tissue. This results in a characteristic "hair-on-end" appearance, particularly visible in the skull. This appearance is caused by the prominence of the diploic spaces and the associated periosteal reaction.[65,66]

Growth abnormalities in children with SCD may present as delayed or abnormal skeletal growth. X-rays can reveal discrepancies in bone length, abnormal growth plate development, or skeletal deformities such as “fish mouth” vertebrae, kyphosis, rib deformities, and changes in long bone alignment/curvatures. Joint abnormalities resulting from repeated vaso-occlusive episodes and AVN, may cause joint space narrowing, subchondral cysts, and changes in joint contours.[67]

Cortical thinning and pathological fractures can also be observed on X-rays, showing thinning of the cortical bone and increased risk of fractures due to compromised bone integrity.

Magnetic Resonance Imaging (M.R.I.)

MRI is highly effective in evaluating musculoskeletal complications in SCD, providing detailed images of both bone and soft tissue. One of the key areas where MRI excels is in the detection of bone infarcts and AVN. Early and subtle changes associated with AVN are often visible on MRI. This includes bone marrow edema, which appears as increased signal intensity on T2-weighted images and fat-saturated sequences, indicating edema within the affected bone marrow. This edema is frequently one of the earliest signs of AVN. As the condition progresses, MRI can reveal subchondral bone collapse and associated joint space narrowing, with areas of low signal intensity on T1-weighted images and high signal intensity on T2-weighted images. The T2-blackout effect on MRI Diffusion Weighted Imaging shows promise as a useful tool in early detection of bone infarcts. This effect is characterized by dark signals on the DWI image due to erythrocyte sickling and sequestration in the bone, indicating marrow infarction. Additionally, MRI detects the replacement of normal marrow with necrotic tissue, showing low signal intensity on T1-weighted images and high signal intensity on T2-weighted images, often with a characteristic “double line sign” indicating granulation tissue at the boundary of necrotic bone.[68–70]

When it comes to sickle cell osteomyelitis, MRI provides detailed images of infection-related changes. It shows increased signal intensity on T2-weighted and fat-saturated images, reflecting marrow edema and inflammation. There may also be areas of low signal intensity on T1-weighted images corresponding to abscess formation or necrotic tissue. Furthermore, MRI can visualize associated soft tissue changes, including periosteal thickening and soft tissue edema, which help to highlight the extent of the infection.[71,72]

The differentiation between bone infarcts and osteomyelitis is critical, and MRI plays a pivotal role in this distinction. On MRI, these infarcts are typically characterized by the "double line sign", which is visible on T2-weighted images and indicates a central area of necrosis surrounded by a reactive zone of bone. Additionally, bone infarcts often present with well-demarcated areas of marrow signal abnormality, showing low signal intensity on T1-weighted images and high signal intensity on T2-weighted images due to associated edema or reactive changes. Importantly, infarcts do not usually disrupt the cortical bone or involve soft tissues, making their identification more straightforward. On the other hand, MRI findings for osteomyelitis include diffuse bone marrow edema that appears low on T1-weighted images and high on T2-weighted images, similar to infarcts. However, osteomyelitis is marked by significant cortical involvement and periosteal reactions, with disruptions in the bone cortex indicating infection. Moreover, the presence of soft tissue involvement is a key differentiator; MRI can show elevated signal intensity in the surrounding tissues due to infection, including abscess formation or fluid collections, which are strong indicators of osteomyelitis. The overlap of features between these two conditions can pose diagnostic challenges. Both bone infarcts and osteomyelitis may present with bone marrow edema, but MRI's detailed imaging allows for the assessment of periosteal reactions and soft tissue changes that are more characteristic of osteomyelitis. In some cases, bone infarcts can become secondarily infected, leading to a complex clinical presentation that MRI can help clarify by revealing features of both conditions.[73,74]

Marrow hyperplasia due to increased hematopoiesis is another important finding on MRI. The marrow may appear hyperintense on T1-weighted images due to increased hematopoietic tissue, and this is often accompanied by decreased signal intensity on T2-weighted images compared to normal fatty marrow.[75] For evaluating joint abnormalities, MRI is sensitive to early changes in joint integrity. It can detect cartilage damage and changes in the subchondral bone, showing areas of degeneration or early osteoarthritis. MRI is also useful in assessing structural changes related to bone and joint deformities. It provides detailed images of bone and joint alignment, helping to assess and quantify

deformities such as kyphosis, scoliosis, or joint misalignment. Additionally, MRI is effective in identifying pathological fractures. It visualizes subtle fracture lines and associated bone marrow edema, which might not be as clearly visible on X-rays.[76,77]

Nuclear Imaging

Nuclear imaging, including techniques such as bone scintigraphy and PET, provides valuable insights into the musculoskeletal complications associated with SCD.

Bone scintigraphy, or bone scan, typically reveals increased radiotracer uptake in areas affected by acute vaso-occlusive crises or infection. This heightened uptake is due to increased bone metabolism and blood flow in these regions, appearing as focal areas of increased activity on the scan. Conversely, in cases of bone infarcts or AVN, bone scintigraphy might show decreased radiotracer uptake in the affected areas. This decreased uptake reflects reduced blood flow and altered bone metabolism in necrotic regions, which often appear as cold spots compared to the surrounding normal bone. A "double ring" sign is often seen in delayed scans of osteonecrosis, which signifies a peripheral zone of increased uptake (representing hyperemia) surrounding a central cold area. An increased radiotracer uptake in all 3 phases (blood flow, blood pool, and delayed) indicate infection, showing localized heightened blood flow and metabolism in both the infected bone and surrounding soft tissues. The differential uptake helps to differentiate bone infarcts from osteomyelitis.[78–82] PET scan, particularly using FDG, highlights increased glucose metabolism in areas of infarction, active inflammation, infection, or bone marrow hyperplasia. Regions of high FDG uptake indicate active disease processes. PET scans can also reveal increased FDG uptake in areas of marrow hyperplasia, reflecting elevated metabolic activity due to increased hematopoiesis. Additionally, PET imaging provides a detailed assessment of inflammatory changes associated with osteomyelitis, helping to evaluate active disease in bone and surrounding soft tissues.[83]

Hematologic Alterations in SCD

Hemolytic anemia is a fundamental and pervasive issue in SCD, marked by the excessive breakdown of RBCs. This destruction releases large amounts of free hemoglobin into the bloodstream. Once in circulation, this free hemoglobin binds with nitric oxide, a molecule crucial for vasodilation—the process by which blood vessels widen to increase blood flow. The binding of hemoglobin to nitric oxide impairs this vasodilatory function, leading to several significant complications [52]. For instance, the reduced ability of blood vessels to dilate can contribute to the development of pulmonary hypertension, which results in excessive strain on the heart and impaired respiratory function.[56] Similarly, it can lead to the formation of leg ulcers, painful open sores that are difficult to heal due to impaired blood flow.[84] Chronic hemolytic anemia also exerts a broader impact on the body. The persistent deficiency of circulating red cell mass means that oxygen delivery to tissues is compromised, resulting in fatigue, which affects daily functioning and overall quality of life. In children, this anemia can slow growth and development, leading to delays in reaching developmental milestones. Moreover, chronic oxygen deprivation from hemolysis leads to chronic ischemia, leading to damage and impaired function of various organ systems over time.[85]

Eryptosis, which refers to the programmed cell death of RBCs, is accelerated in SCD due to several interconnected mechanisms that play a significant role in worsening the effects of SCD. Increased intracellular calcium, driven by heightened RBC membrane permeability, activates calpain and phospholipid scrambling, leading to cell shrinkage and phosphatidylserine exposure. Oxidative stress from hemoglobin breakdown and chronic hemolysis exacerbates this process by damaging membrane components and enhancing calcium entry. Ceramide, formed through sphingomyelinase activity, further sensitizes the membrane to calcium and promotes phosphatidylserine externalization. Chronic inflammation and reduced ATP availability also impair cellular functions and contribute to early RBC death. The excessive eryptosis in SCD exacerbates anemia, promotes microcirculatory blockage due to phosphatidylserine-exposed RBCs adhering to endothelial cells, and increases the risk of vaso-occlusive crises, thrombosis, reduced tissue oxygenation, and associated complications.[86–88]

Vaso-occlusive crises in SCD result from the sickling of RBCs that adhere to blood vessel walls, causing painful episodes and severe complications like acute chest syndrome, stroke, AVN of bones, and splenic infarction. These crises lead to long-term tissue ischemia and chronic organ damage.[49] Increased RBC adhesion and altered blood rheology contribute to vascular blockages, resulting in ischemic strokes and silent cerebral infarctions, which can cause persistent neurocognitive issues. Hematopoietic alterations in SCD lead to ineffective erythropoiesis, chronic anemia, and bone marrow expansion, causing splenomegaly, functional asplenia, increased infection risk, pain, and osteopenia.[89] The disease also induces a hypercoagulable state due to endothelial damage, platelet activation, and chronic inflammation, increasing the risk of VTE, DVT, and stroke, and exacerbating acute chest syndrome. Chronic inflammation from ongoing hemolysis and endothelial dysfunction worsens conditions like pulmonary hypertension, chronic kidney disease, and priapism, and can lead to liver cirrhosis and heart failure. Additionally, oxidative stress from free hemoglobin and heme damages endothelial cells, contributing to acute chest syndrome, chronic lung disease, and silent cerebral infarctions, which can affect cognitive function and learning in children.[90] D-dimer serves as a vital biomarker for predicting acute complications like vaso-occlusive crises and for prognosticating long-term risks such as stroke, pulmonary hypertension, and organ dysfunction.[91]

Elevated D-dimer levels during vaso-occlusive crises indicate increased thrombin generation and fibrin formation, reflecting a hypercoagulable state.[92] Furthermore, high D-dimer levels are associated with an increased risk of stroke and pulmonary hypertension, making it a valuable prognostic marker for these severe complications.[93,94]. D-dimer also provides insights into organ dysfunction, such as hepatic and renal impairment, and has been linked to higher mortality risks in SCD patients.[95,96]

Sickle Cell Nephropathy (SCN)

SCN is a major complication of SCD, encompassing a wide range of renal pathologies emanating from chronic vaso-occlusion, hemolysis, and metabolic disturbances. SCN development is influenced by several associated factors, particularly in early childhood. Renal complications often begin in infancy, marked by hyperfiltration as a compensatory response to anemia, leading to increased renal blood flow and glomerular filtration rate. While this may initially maintain kidney function, prolonged hyperfiltration ultimately leads to nephron loss and progression to CKD. Prostaglandins and nitric oxide, released in response to ischemia, mediate renal blood flow and vasodilation, but persistent hyperfiltration is detrimental over time.[98,102,106]

As children age, many experience hyposthenuria, characterized by an inability to concentrate urine, which results in nocturia and enuresis, impacting quality of life. The severity of anemia, closely tied to hemolysis levels (as evidenced by elevated LDH and low haptoglobin), directly affects renal function; while acute compensatory hyperfiltration may occur initially, persistent hyperfiltration ultimately leads to glomerular injury and worsened renal outcomes. Free hemoglobin released during hemolysis further contributes to renal damage by inducing oxidative stress, apoptosis, and dysfunction in podocytes, culminating in glomerulosclerosis and interstitial fibrosis. Additionally, endothelial dysfunction, characterized by chronic inflammation and elevated markers of vascular injury, disrupts the renal microvasculature and promotes ischemia, exacerbating the "perfusion paradox," where systemic blood flow is maintained despite renal hypoperfusion, thus increasing the risk of ischemia/reperfusion injury to the kidneys.[97,98]

Multiple molecular mechanisms that contribute to renal injury, are primarily driven by vaso-occlusion and ischemic processes. Severe sickling of erythrocytes, lead to vaso-occlusive crises, particularly affecting the hypoxic-sensitive renal medulla. These crises precipitate ischemic insults that trigger a cascade of cellular events, resulting in renal tubular atrophy and glomerulosclerosis. The transition from ischemia to reperfusion activates pro-inflammatory cytokines and oxidative stress pathways, further exacerbating renal cell damage. This cycle perpetuates renal dysfunction, creating a vicious spiral of injury.[99,100]

Glomerular pathology in SCN is predominantly characterized by focal segmental glomerulosclerosis (FSGS), which results from increased intraglomerular pressure and podocyte injury caused by free hemoglobin. Damage to the glomerular filtration barrier—including podocytes, endothelial cells, and the basement membrane—leads to increased proteinuria, reflecting the extent of glomerular injury. Chronic hemolysis exacerbates endothelial dysfunction, reducing nitric oxide levels and increasing vascular permeability, facilitating albumin leakage into urine. This reflects a broader pattern of vascular damage that underpins renal impairment.[100–103]

Tubular dysfunction is another critical aspect of renal injury in SCN. Acute tubular injury, driven by elevated ROS during hemolytic crises, promotes apoptosis and disrupts renal tubular integrity. The proximal tubules are particularly vulnerable, leading to tubular atrophy and impaired function. The combination of glomerular and tubular injuries significantly impacts renal function, often indicated by elevated serum creatinine levels. However, early changes may not be readily apparent due to compensatory hyperfiltration mechanisms.[104,105]

Markers of hemolysis, such as elevated free hemoglobin and low hemopexin levels, indicate ongoing renal damage. Additionally, urine iron markers like hemosiderin and hemoglobinuria reflect the toxic effects of free hemoglobin on tubular cells, contributing to oxidative stress and cellular apoptosis. The nitric oxide scavenging effect of free hemoglobin exacerbates endothelial dysfunction and vasoconstriction, further compromising renal blood flow.[106,107]

The pro-inflammatory state prevalent in SCD is characterized by elevated levels of endothelin-1 (ET1) and soluble urokinase-type plasminogen activator receptor (suPAR). ET1 contributes to renal microvascular injury through vasoconstriction and glomerular hypertension, accelerating glomerulosclerosis. The rising levels of suPAR are indicative of sustained inflammation and correlate with the incidence of FSGS, highlighting the

interplay between inflammation and renal pathologies in sickle cell disease. Together, these mechanisms illustrate the multifaceted nature of renal injury in SCN.[108,109]

Proteinuria, particularly assessed through the urinary albumin-creatinine ratio (ACR), is a critical marker for renal injury. An ACR ≥ 30 mg/g indicates microalbuminuria, while ACR ≥ 300 mg/g signifies macroalbuminuria, both serving as early predictors of CKD progression and ESRD. The estimated glomerular filtration rate (eGFR), commonly derived from serum creatinine or cystatin-C, provides essential insights into renal function, with a rapid decline in GFR (>3-5 mL/min/1.73 m² per year) indicating heightened risk for CKD progression. Serum creatinine remains the conventional measure of renal function, reflecting a degree of loss of nephron functioning, cystatin-C offers a more accurate assessment, especially in pediatric populations, allowing for earlier detection of renal impairment. Additionally, markers of hemolysis such as free hemoglobin and low hemopexin levels indicate active hemolysis and correlate with renal injury severity, serving as predictors for acute kidney injury (AKI) and CKD progression during vaso-occlusive crises. Lastly, markers of inflammation, including ET1 and suPAR, highlight endothelial activation and vascular injury, with elevated levels associated with increased renal damage and the potential for CKD progression. Together, these biomarkers underscore the importance of comprehensive renal monitoring in patients with SCD.[108,110–114 109]

Cardiovascular Dysfunction in SCD

Systolic and diastolic dysfunction significantly contribute to the morbidity and mortality associated with cardiovascular complications in patients with sickle cell disease (SCD). Chronic anemia, hemolysis, and vascular damage increase the heart's workload, ultimately leading to heart dysfunction. Systolic dysfunction in SCD is characterized by the heart's compensatory mechanisms in response to chronic anemia, which force an increase in cardiac output. Over time, this leads to left ventricular dilation and eccentric hypertrophy, impairing myocardial contractility. While traditional measures like ejection fraction may appear normal, they often do not reflect true systolic function, necessitating the use of load-independent indices like end-systolic stress to end-systolic volume index for a more accurate assessment.[115–117]

Diastolic dysfunction is more prevalent in SCD patients, affecting up to 18% of them. It is primarily marked by impaired left ventricular relaxation and increased filling pressures, driven by chronic myocardial ischemia due to microvascular occlusion and inflammation. Iron overload from repeated blood transfusions exacerbates myocardial damage. Diastolic dysfunction is often evaluated using tissue Doppler imaging or the E/A ratio, with lower values indicating significant impairment. Notably, there is a strong association between diastolic dysfunction and pulmonary hypertension, which can further increase mortality risk among SCD patients.[118–120]

The underlying molecular mechanisms of cardiovascular dysfunction in SCD include chronic hemolysis and vaso-occlusion. Hemolysis releases free hemoglobin into the bloodstream, reducing nitric oxide availability, leading to vascular constriction and endothelial dysfunction, which promotes pulmonary hypertension and increases the cardiac workload. Additionally, iron overload from frequent blood transfusions can lead to myocardial siderosis, damaging cardiac tissues and impairing both systolic and diastolic function.[117]

N-terminal pro-brain natriuretic peptide (NT-proBNP) is an essential biomarker in cardiovascular diseases, particularly for predicting and assessing complications of SCD. NT-proBNP is released in response to pressure or volume overload in the heart, serving as an indicator of increased cardiac stress. High levels of NT-proBNP are commonly associated with conditions such as pulmonary hypertension and left ventricular dysfunction.[121,122]

As a reliable predictor of mortality in SCD, NT-proBNP levels are strongly correlated with increased risk of death in both children and adults. In adults, NT-proBNP levels exceeding 160 ng/L are associated with a significantly increased mortality risk, exceeding a relative risk of 6.0. The severity of pulmonary hypertension, which can elevate risk of early death significantly, is also reflected in NT-proBNP levels. This makes NT-proBNP a critical marker for monitoring patients with SCD.[123,124]

NT-proBNP levels correlate closely with the severity of hemolytic anemia in SCD. Chronic hemolysis releases free hemoglobin, reducing nitric oxide availability and leading to vascular damage, which increases cardiac stress reflected by elevated NT-proBNP levels. Other biomarkers, such as lactate dehydrogenase (LDH), bilirubin, and reticulocyte counts, are also linked to NT-proBNP, indicating the underlying hemolytic stress driving cardiovascular complications. Additionally, renal markers like blood urea nitrogen (BUN) and creatinine are associated with higher NT-proBNP, suggesting that renal dysfunction contributes to cardiovascular stress in SCD patients.[125,126]

NT-proBNP provides a prognostic value that is independent of other clinical measures, including hemoglobin levels and pulmonary pressure markers. Its ability to reflect dysfunction in both the right and left ventricles makes it a crucial tool for identifying high-risk patients. NT-proBNP is a superior biomarker for assessing cardiovascular stress and heart failure compared to other markers.[127] While traditional indicators like LDH and bilirubin primarily reflect hemolysis, NT-proBNP is more closely associated with mortality, especially in patients with pulmonary hypertension[123]. When used alongside markers such as LDH and BUN, NT-proBNP helps create a comprehensive understanding of cardiovascular and hemolytic stress in sickle cell disease (SCD), facilitating the identification of patients at heightened risk for early mortality. Additionally, the severity of hemolysis is correlated with various cardiopulmonary metrics, including left atrial and left ventricular dimensions, as well as lung function measures like oxygen saturation and lung capacities. This underscores the importance of both hemolysis and anemia in predicting the degree of cardiovascular dysfunction.[126]

Despite its utility, there are limitations to consider when using NT-proBNP. Levels are generally higher in children, necessitating age-specific thresholds for accurate risk assessment. In addition, renal dysfunction, which is common in SCD, can elevate NT-proBNP levels, making it important to differentiate between cardiac and renal causes of increased levels in patient evaluations.[124]

Determinants of SCD Severity and Complications

The hemoglobin profile is crucial for assessing anemia severity in SCD. Lower total hemoglobin levels indicate greater hemolysis, leading to severe symptoms and an increased risk of complications such as stroke and ACS. Chronic hemolysis also raises bilirubin levels, which can result in gallstones and complicate patient management. HbF plays a protective role by inhibiting the polymerization of sickle hemoglobin, reducing the sickling of red blood cells. Patients with higher HbF levels tend to experience fewer painful crises and demonstrate improved survival rates, especially those with HPFH.[128,129]

Platelet indices, including platelet count and mean platelet volume (MPV), are important for understanding the thrombotic risks in SCD. Thrombocytosis, or elevated platelet counts, is common in SCD and often correlates with disease activity; higher platelet counts may indicate greater inflammation and an increased risk of thrombotic events, such as stroke and ACS. MPV serves as a marker of platelet activation, with increased levels indicating heightened reactivity. In SCD, elevated platelet counts and MPV suggest a more severe disease state and predispose patients to complications related to vascular occlusion.[130]

The reticulocyte count is another vital marker in SCD, representing the number of immature red blood cells released in response to anemia. An elevated reticulocyte count indicates increased erythropoietic activity due to hemolysis, often correlating with severe anemia and a heightened risk of complications like ACS and vaso-occlusive crises. This count reflects the bone marrow's compensatory response to ongoing hemolytic stress, making reticulocyte levels crucial for assessing disease severity.[129,131]

Endothelial dysfunction plays a significant role in vascular complications in SCD. Soluble Vascular Cell Adhesion Molecule-1 (sVCAM-1) serves as a marker of endothelial injury, with high levels indicating increased risks of vaso-occlusive crises. The release of free hemoglobin during hemolysis decreases NO availability, leading to

vasoconstriction and complications like pulmonary hypertension. Asymmetric Dimethylarginine (ADMA), an inhibitor of nitric oxide synthase, rises in SCD, reflecting reduced NO availability and endothelial dysfunction.[19,132]

Patients with SCD are often in a hypercoagulable state, heightening thrombotic risks. D-Dimer, a marker of clot breakdown, tends to be elevated during acute events, indicating an ongoing hypercoagulable state. Prothrombin Fragment 1+2 (F1+2) reflects thrombin generation and is similarly elevated, indicating increased thrombosis risk. Fibrinogen, an acute-phase protein, also rises during crises, indicating increased thrombotic potential and reflecting the inflammatory response. High homocysteine levels also have been implicated as a contributing factor in the hypercoagulable state in SCD.[94,96,133]

Oxidative stress contributes to cell damage and inflammation in SCD. Malondialdehyde (MDA), a marker of lipid peroxidation, indicates oxidative damage and is associated with hemolysis. Elevated MDA levels suggest significant oxidative stress in SCD patients. Glutathione (GSH), a key antioxidant, is often reduced in SCD, leading to increased oxidative damage, hemolysis, and overall disease severity.[134] Hypocholesterolemia in SCD is associated with chronic hemolysis and inflammation, leading to alterations in HDL particles that may impair lipid metabolism and increase the risk of systemic inflammation and cardiovascular complications.[135] .

Urinary ACR serves as a sensitive marker for kidney injury, with elevated levels indicating proteinuria and glomerular damage. Serum Creatinine levels provide insights into kidney function, with higher levels suggesting impaired renal health. Cystatin C, less influenced by muscle mass, serves as a reliable indicator of renal function, with elevated levels indicating reduced glomerular filtration rate and chronic kidney disease.[136]

Other urinary biomarkers can offer insights into specific organ complications in SCD. Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL) is a marker for acute

kidney injury, with elevated levels indicating renal stress during acute crises. Urine Microalbumin is an early indicator of kidney damage, with higher levels suggesting increased risk for nephropathy and CKD.[131,136]

Hydroxyurea

Hydroxyurea (HU) is a drug recognized for its diverse therapeutic applications, particularly in the management of SCD. With a chemical formula of $CH_4N_2O_2$, HU's structure features a hydroxyl group (-OH) attached to a urea core. This structural configuration results in a highly polar and water-soluble compound, which facilitates its rapid absorption when administered orally. The drug's solubility and absorption characteristics play a critical role in its pharmacodynamics, allowing it to effectively interact with specific enzymes in the body, especially ribonucleotide reductase (RNR). This enzyme is pivotal for DNA synthesis, and hydroxyurea's action on RNR is central to its therapeutic effects, especially in the hematopoietic tissues.[137]

In the realm of SCD management, HU is distinguished as the most widely employed disease-modifying therapy. The primary mechanism through which hydroxyurea exerts its therapeutic effects is by increasing the levels of HbF in the RBCs. HU acts primarily by inhibiting RNR, an enzyme critical for converting ribonucleotides to deoxyribonucleotides, which are essential for DNA synthesis. This inhibition decreases the availability of deoxyribonucleotides, creating stress in erythroid progenitor cells in the bone marrow, prompting them to produce HbF instead of HbS. Even modest increases in HbF significantly reduce sickling and vaso-occlusive events in SCD patients. In addition to enhancing HbF production, hydroxyurea improves red blood cell rheology, decreases inflammation, and raises nitric oxide levels, which helps with vasodilation and improves blood flow. Its inhibition of RNR also makes hydroxyurea effective as an antineoplastic agent by targeting rapidly dividing cells, such as those in cancer. While the precise mechanism behind HbF induction is not fully understood, it is believed to involve stress erythropoiesis and nitric oxide signaling.[138–140]

While hydroxyurea is an effective treatment for various conditions, its potential adverse effects must be carefully considered as they can impact patient adherence and overall treatment success. A major side effect is myelosuppression, characterized by decreased bone marrow activity leading to leukopenia, thrombocytopenia, and anemia, necessitating regular blood count monitoring and possible dosage adjustments.[137,141] Gastrointestinal side effects, including nausea, vomiting, and diarrhea, are common but typically mild and transient, yet they can deter patients from continuing therapy.[142] Skin-related toxicities such as ulcers, hyperpigmentation and even dermatomyositis, although relatively rare and manageable, can arise, particularly with long-term use.[143,144] A more serious concern is the potential association between chronic hydroxyurea use and malignancies, especially squamous cell carcinoma, with some studies suggesting increased risk in patients with prolonged exposure.[145,146] Additionally, hydroxyurea can adversely affect male reproductive health by reducing sperm counts, which necessitates discussions about fertility among male patients, especially younger individuals, although the effects do seem to reverse after discontinuation of the therapy.[26,147] The drug's teratogenic potential also raises concerns for pregnant women, and while it is generally avoided during pregnancy unless absolutely necessary, the long-term effects of fetal exposure are still under investigation.[148]

Aims & Objectives

Aim

The performance of hematological and imaging parameters in predicting adverse outcomes including vaso-occlusive sickle cell crises and chronic complications in adult sickle cell anemia.

To fulfil our aim, we have the following objectives:

Objectives

1. To correlate Hematological and biochemical parameters (Hemoglobin level, Hemoglobin variants on electrophoresis) in adults with Sickle cell anemia with frequency of acute crises in one year and chronic complications.

2. To compare Imaging (radiological / bone scan) in adult Sickle cell anemia with the frequency of acute crises in one year and chronic complications.

3. To correlate imaging (radiological / bone scan) with hematological parameters (Hemoglobin levels, Hemoglobin variants on electrophoresis).

Materials & Methods

Study design: We planned a cross-sectional, observational study.

Setting: Patients with Sickle cell anemia who were in follow-up of the Medicine and Hematology Outpatient department (OPD), AIIMS BHOPAL in the years 2023 and 2024, are recruited.

Ethics statement: We identified eligible participants from the Medicine and Hematology Outpatient Department (OPD), AIIMS BHOPAL. We conducted one questionnaire-based interview with the eligible participants, for which we sought written informed consent from the participants. All of the study procedures were initiated after obtaining a letter of permission from the Ethics committee of the institute.

Operational definition:

1. Acute Painful Crisis -

   An acute painful crisis is defined as an acute episode of pain in patients with sickle cell disease that meets all of the following criteria:

   a. Duration: The pain episode must last for at least 1 day (24 hours).
   b. Cause: The pain must have no identifiable cause other than a vaso-occlusive event (i.e., no other known medical conditions or factors causing the pain).
   c. Treatment Requirement: The pain must require treatment with parenteral or oral narcotics (including opiates) or parenteral NSAIDs (e.g., intravenous or intramuscular nonsteroidal anti-inflammatory drugs).

2. Hospitalization -

   A hospitalization for the purpose of the study is defined as an inpatient admission to the hospital for a duration of at least 24 hours due to acute symptoms or complications directly associated with sickle cell disease. This includes, but is not limited to:

- Vaso-occlusive Crisis (VOC)
- Acute Chest Syndrome (ACS)
- Severe Anemia
- Infection

3. Dosage -

The dose of medication in this study is defined as the daily amount of hydroxyurea taken by each patient, expressed in milligrams per kilogram of body weight per day (mg/kg/day), and used to categorize patients into two groups based on the following criteria:

a. Low Dose Group: Patients who are prescribed and consistently take less than or equal to 25 mg/kg/day of hydroxyurea.

b. High Dose Group: Patients who are prescribed and consistently take more than 25 mg/kg/day of hydroxyurea.

Formula:
Dose (mg/kg/day)= Total daily dose of hydroxyurea (mg) / Patient's body weight (kg)

4. Compliance-

Compliance is assessed by determining whether the patient takes the prescribed dose of hydroxyurea and folic acid daily as instructed by their healthcare provider.

The period of observation for compliance will be 12 months, which was further broken down into quarterly reviews, during which medication intake was monitored.

Data collection was done by counting the number of pills left in the medication blister (packet) during the follow-up visit.

A patient was considered compliant if they took at least 80% of the prescribed medication (Hydroxyurea/ Folic Acid) doses during the study period.

Non-compliance will be defined as taking fewer than 80% of the prescribed doses within the same time frame.

Any intentional discontinuation of hydroxyurea based on a healthcare provider's advice due to side effects or other clinical reasons was not counted against the compliance rate.

Formula for Compliance:

Compliance (%) = (Number of days medication was taken as prescribed / Total number of days in observation period) ×100.

5. Health Education Frequency-

It is defined as the number of times an adult patient with sickle cell disease receives structured health education sessions about managing their condition over 12 months. Based on the number of sessions attended or provided, patients are categorized into one of the following three groups:

a. Never: Patients who did not receive or attend any health education sessions during the study period.

b. Occasional: Patients who received or attended 1 to 3 health education sessions during the study period.

c. Frequent: Patients who received or attended 4 or more health education sessions during the study period.

Educational sessions may include information on managing pain crises, medication adherence (e.g., hydroxyurea), hydration, nutrition, and recognizing complications of sickle cell disease.

Participants:

We included the following participants in the study

- Adults (>18 years of Age) with Sickle Cell Anaemia. (HbS present in Hb HPLC report)

We excluded the following patients

- Patients who deny consent for participation in the study
- Patients who were Pregnant / Lactating during the study duration
- Patients who were admitted with critical care illness or acute crisis episodes

Procedure:

We started the study after ethical clearance from the institutional ethics committee. All adult patients with Sickle Cell Disease coming to Medicine and Hematology OPD were approached regarding participation in the study. Informed consent was obtained from all participants.

All eligible and consenting participants were included in the study.
The following steps were performed.

1. A structured questionnaire based history was elicited,history along with demographic details (hospital ID no, age, gender, district, socioeconomic strata) (The socioeconomic strata was divided into Above Poverty Line - APL and Below Poverty line - BPL), along with Anthropometry data (Height and Weight) and vitals (Pulse and Blood Pressure)

2. Patients diagnosed with Sickle cell disease were asked for a detailed history and an examination was done for

    a. Painful crisis -
        - Frequency of Sickle cell painful crisis events (in 12 months),
        - Duration of average painful event (in days) (arithmetic mean calculated of all painful crisis events in 12 months),
        - Severity of pain was noted using a Wong-Baker FACES Pain Rating Scale for which was most frequently experienced by the patient (in 12 months),

    b. Health care visits -
        - Frequency of hospitalization in 12 months
        - Type of Health care facility (Primary center, Secondary center, Tertiary center, Private Hospital or Private clinic) (First and most frequent contact)
        - Most frequent reason for hospitalization (Pain, Weakness, Fever, Other)

    c. Blood Transfusion (In 12 months)
        - Blood Transfusion frequency,
        - Cumulative units of PRBC transfused.

    d. Drug history at the time of enrollment (In the last 12-month period) -
        - Hydroxyurea (Yes/No)
            - If Yes, Dosage (High Dose, Low Dose)
        - Folic Acid (Yes /No)
        - Compliance (Yes/No)

    e. Health Education (Regular, Occasional, Never)

3. These patients then had routine blood and urine examinations done and recorded.

    a. Hematological parameters:
        - Hemoglobin (Hb)
        - Total Leucocyte count (TLC)
        - Neutrophil percentage (P%)
        - Lymphocytes percentage (L%)
        - Platelets (PLT)
        - Reticulocyte count (RETIC)
        - HbHPLC was analyzed following chromatography using BIO-RAD 10 HEMOGLOBIN TESTING SYSTEM, and the following was recorded
            - Percentage of HbS by HbHPLC (HbS)
            - Percentage of HbA0 by HbHPLC (HbA)
            - Percentage of HbA2 by HbHPLC (HbA2)
            - Percentage of HbF by HbHPLC (HbF)
        - D dimer - Indicator of hypercoagulability

Figure 2 : BIO-RAD D-10 Hemoglobin Testing System [149]

Source :https://www.bio-rad.com/en-in/sku/2200600-d-10-rack-loader?ID=2200600

The HbHPLC was interpreted as follows:[150]

(a) HbSS - HbS - >50%
HbA - <10%
HbA2 - <3.5%
HbF - >5%

(b) HbAS - HbS - >50%
HbA - 50-60%
HbA2 - <4%
HbF - <1%

(c) Hbβ^{+}S - HbS - 60-80%
HbA - 5-30%
HbA2 - >3.5%
HbF - >5%

(d) Hbβ^{0}S - HbS - 80-90%
HbA - <5%
HbA2 - >3.5%
HbF - >5%

b. Biochemical parameters:
- Renal function markers (Blood Urea, Serum Creatinine, Urine ACR)

Urine Microalbuminuria (Urine Albumin Creatinine Ratio) was further categorized as per follows :

Normal: Less than 30 mg/g
Microalbuminuria: 30-300 mg/g
Macroalbuminuria: Greater than 300 mg/g

- Cardiac function markers (BNP)

c. Imaging parameters:

- Avascular Necrosis -

Ficat and Arlet classification (Xray, MRI, Bone Scan)[72]

All patients underwent a screening X RAY PELVIS with Both Hips, and an MRI of the joint was done for participants who had complaints suggestive of pain and/or stiffness of any joint.

A Tc99 Triple phase Bone Scan was performed for participants for whom the test was feasible.

Classified as follows : Stage 1: Normal X-ray; MRI shows edema,Stage 2: Sclerosis and cystic changes on X-ray without collapse;Stage 3: Subchondral fracture with a "crescent sign" and early collapse;Stage 4: Joint space narrowing, secondary osteoarthritis, and femoral head collapse.

- 2D ECHO -

A comprehensive 2D ECHOCARDIOGRAPHY examination was performed in all patients.

i. Diastolic dysfunction -[151]

The following steps were used to assess the diastolic dysfunction:

<u>Measurement of E-wave (Early Diastolic Filling Velocity):</u>

-The velocity of blood flow through the mitral valve during the early passive filling phase of the left ventricle is measured as the E-wave using pulsed-wave Doppler echocardiography.

-Record the E-wave velocity for each subject.

<u>Measurement of A-wave (Atrial Contraction/Active Filling):</u>

-The velocity of blood flow through the mitral valve during atrial contraction (the active filling phase) is measured as the A-wave.

-Record the A-wave velocity for each subject.

<u>Calculation of E/A Ratio:</u>

Calculate the E/A ratio by dividing the E-wave velocity by the A-wave velocity.

Interpretation of E/A ratio:

1. Normal diastolic function:
   E/A ratio between 1.0 and 1.5.
2. Mild diastolic dysfunction (impaired relaxation):
   E/A ratio < 1 (E-wave decreases, A-wave increases).
3. Moderate diastolic dysfunction:
   E/A ratio > 1, as LV stiffness causes the E-wave to increase and the A-wave to decrease.
4. Severe diastolic dysfunction (restrictive filling):
   E/A ratio > 2, with a significantly higher E-wave compared to the A-wave.

ii. Systolic dysfunction -[152]

The following steps were used to assess the systolic dysfunction:

Measurement of LVIDd (Left Ventricular Internal Diameter in Diastole):

- In the parasternal long axis (PLAX) view, measure the LVIDd at the end of the diastole when the left ventricle is fully relaxed and filled with blood.
- The measurement is taken at the level of the mitral valve leaflet tips, just before the ventricle starts to contract.
- This measurement reflects the size of the left ventricle at its largest point.

Measurement of LVIDs (Left Ventricular Internal Diameter in Systole):

- In the PLAX view, measure the LVIDs at the end of the systole, when the ventricle has completed its contraction and is at its smallest size.
- The measurement is taken at the same level as LVIDd when the mitral valve is closed and the ventricle has ejected the blood.

Calculation of Ejection Fraction (EF):

- Use the measured LVIDd and LVIDs to estimate ejection fraction (EF), which is the percentage of blood pumped out of the left ventricle during systole.

- Formula (using M-mode echocardiography):

$$EF\ (\%) = [(LVIDd^3 - LVIDs^3) \div LVIDd^3] \times 100$$

Interpretation of LV Systolic Dysfunction Based on Ejection Fraction (EF):

1. Normal Function: EF ≥ 55%
2. Mild Systolic Dysfunction: EF 45-54%
3. Moderate Systolic Dysfunction: EF 30-44%
4. Severe Systolic Dysfunction: EF < 30%

d. Others -

PFT -

Patients with complaints of dyspnea (mMRC grade ≥ 2), despite hemoglobin optimization to ≥ 7 g/dL, were selected for further assessment.

A pulmonary function test (PFT) was performed using spirometry to evaluate lung function.

Spirometry results were classified into the following patterns based on standard criteria:

a. Normal: Both the Forced Vital Capacity (FVC) and Forced Expiratory Volume in 1 second (FEV1) are within the predicted normal range, and the FEV1/FVC ratio is greater than 0.70.
b. Restrictive pattern: The FVC is reduced (less than 80% of the predicted value), but the FEV1/FVC ratio is normal or increased (≥ 0.70), indicating limited lung expansion.
c. Obstructive pattern: The FEV1 is reduced, and the FEV1/FVC ratio is less than 0.70, indicating airflow obstruction.

Statistical Analysis:

Data Entry and Preparation

Data was initially entered into Microsoft Excel, where each variable was coded numerically, and a code sheet was developed for reference. This coding system ensured consistency and accuracy across variables during data entry and analysis.

Data Cleaning

- The dataset was carefully examined for outliers, duplications, and redundancies to maintain the integrity of the analysis.
- Outliers were identified by visual inspection and statistical methods to confirm they did not represent data entry errors. Where appropriate, extreme values were retained, as they were deemed to reflect real variability within the population.
- Redundant entries were removed to ensure each participant was uniquely represented in the dataset.

Statistical Software and Approach

Data analysis was performed using the R software, version 4.3.2. Given the non-normal distribution of data as confirmed through Shapiro-Wilk tests and visual inspection via histograms and Q-Q plots, non-parametric statistical methods were applied.

Summary Statistics

Data summaries were generated as follows:

- Categorical Variables: Frequencies and percentages were used to describe categorical variables, such as the presence of specific complications, genotype distribution, and frequency of healthcare visits.

- Continuous Variables: Medians and interquartile ranges (IQRs) were calculated for continuous variables, including hematological parameters like hemoglobin levels, total leukocyte counts, and imaging-based measurements such as cardiac function indices. These measures provided a robust summary of central tendency and variability, accounting for the skewed distribution of data.

Analytical Tests for Associations

The relationship between variables was explored using the following statistical tests, each selected based on the type and distribution of the data:

1. Fisher's Exact Test:
    - Fisher's Exact Test was employed to assess the association between two categorical variables, especially given the small sample size of certain groups. This test was chosen for its precision in handling small sample counts, which frequently appeared in subgroup analyses (e.g., presence vs. absence of musculoskeletal complications by genotype).
2. Kruskal-Wallis Rank Sum Test:
    - For comparisons involving continuous variables across more than two groups, the Kruskal-Wallis Rank Sum Test was used. This non-parametric alternative to the ANOVA test is suitable for data that does not assume a normal distribution.
    - This test was applied to analyze differences in hematological parameters across groups defined by pain crisis frequency, hospitalization frequency, and Ficat stages of avascular necrosis.

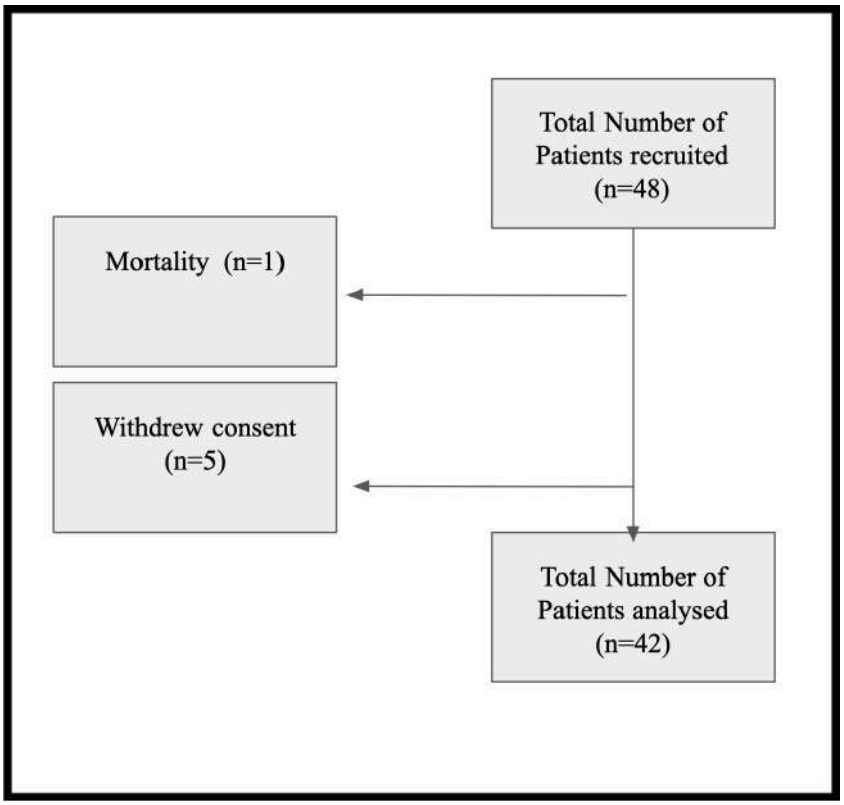
Significance Threshold

A p-value threshold of less than 0.05 was established as the criterion for statistical significance across all analyses. This threshold was applied to results from both Fisher's Exact Test and the Kruskal-Wallis Rank Sum Test. Findings with p-values below this threshold were interpreted as statistically significant, suggesting meaningful associations between variables.

Results

The study started in March 2023 after receiving approval from the Institutional Human Ethics Committee (IHEC), AIIMS Bhopal in February 2023. A total of 48 cases were taken from General Medicine and Medical Oncology & Hematology OPD in this cross-sectional observational study after fulfilling inclusion criteria. Out of this 1 was lost due to mortality and another 5 withdrew consent from the study.

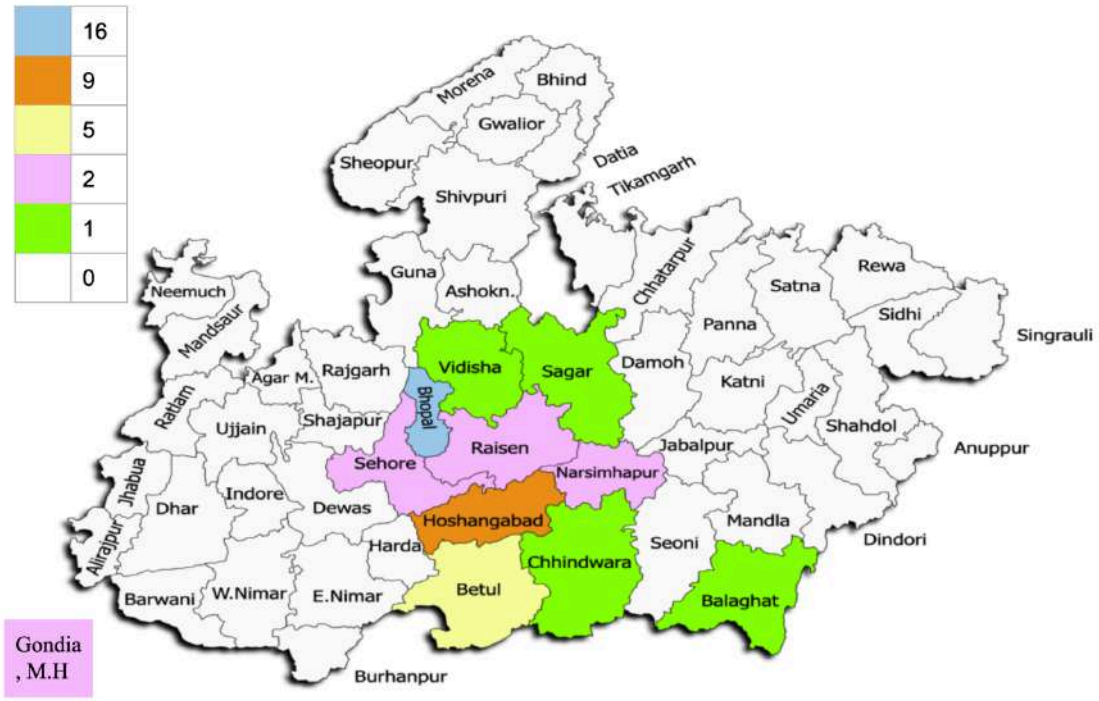
Figure 3 : Study Flow Chart.



The study included a total of 42 participants, with a median age of 25 years, with an Interquartile Range from 20 to 34 years. In terms of gender distribution, the majority were male, comprising 67% (28 individuals), while females accounted for 33% (14 individuals). Participants represented predominantly from the state of Madhya Pradesh (MP) and a few from Maharashtra (MH). Bhopal, MP, was the most represented area with 38.4% (16 participants), followed by Hoshangabad, MP, with 21% (9 participants), and Betul, MP, with 12% (5 participants). Other areas included Narsimhapur, Raisen, and Sehore in MP, as well as Gondia, Maharashtra each accounting for 4.8% (2 participants each), and single representations from Balaghat, Chhindwara, Misrod, Sagar, and Vidisha in MP, each at 2.4% (1 participant).

Socioeconomic status revealed that 64% of the participants were classified as Above Poverty Line (APL), whereas 36% were Below Poverty Line (BPL).

Figure 4: Map showing district wise representation. (Credit: www.paintmaps.com)[153]



Regarding anthropometric measures, the median weight was 47 kg, with an interquartile range (IQR) of 45 to 53 kg, and the median height was 166.0 cm (IQR: 162.3 to 170.0 cm), indicating an overall average body frame. The median Body Mass Index (BMI) was calculated at 17.0, with values spanning from 16.1 to 19.3, suggesting a population on the lower side of normal weight ranges.

Vital signs assessments showed that the median systolic blood pressure (SBP) was 117 mmHg (IQR: 111 to 122 mmHg), while the median diastolic blood pressure (DBP) was 70 mmHg (IQR: 66 to 78 mmHg), indicating blood pressure levels within generally accepted normal ranges. Additionally, the median pulse rate recorded was 84 beats per minute (bpm), with an IQR of 74 to 90 bpm.

Table 1: Demographic details of the participants.

Characteristic	N[1]= 42
Age	25 (20, 34)
Gender	
Female	14 (33%)
Male	28 (67%)
Address	
Balaghat, MP	1 (2.4%)
Betul, MP	5 (12%)
Bhopal, MP	16 (38.4%)
Chhindwara, MP	1 (2.4%)
Gondia,MH	2 (4.8%)
Hoshangabad, MP	9 (21%)
Narsimhapur, MP	2 (4.8%)
Raisen, MP	2 (4.8%)
Sagar, MP	1 (2.4%)
Sehore, MP	2 (4.8%)
Vidisha, MP	1 (2.4%)
Socioeconomic status	
APL (Above Poverty Line)	27 (64%)
BPL (Below Poverty Line)	15 (36%)

[1]Frequency ; n%

Table 2 : Description of anthropometry and vitals of the participants.

Category	Mean[1] (IQR)
Weight (in Kilograms)	47 (45, 53)
Height (in meters)	1.66 (1.62, 1.70)
BMI (in kg/m^2)	17.0 (16.1, 19.3)
Systolic Blood Pressure (in mmHg)	117 (111, 122)
Diastolic Blood Pressure (in mmHg)	70 (66, 78)
Pulse rate (in beats per minute)	84 (74, 90)

1 - Arithmetic mean

The study population experienced varying frequencies of acute pain crisis episodes over the past 12 months. When categorized by frequency, 48% (20 participants) experienced low pain frequency (0-2 episodes), 36% (15 participants) had moderate frequency (3-6 episodes), 12% (5 participants) experienced high frequency (7-12 episodes), and a very high frequency of more than 12 episodes was observed in 4.8% (2 participants).Pain duration among the participants also varied, with a median of 4 days and an interquartile range (IQR) from 2 to 5.75 days. In terms of categorical distribution, 43% (18 participants) reported short pain episodes lasting between 0 to 3 days, while 36% (15 participants) experienced moderate durations of 4 to 6 days. Longer pain durations were reported by 19.4% (8 participants), who experienced episodes lasting 7 to 10 days, and 2.4% (1 participant) reported very long episodes lasting more than 10 days.

Pain severity was similarly distributed across different levels. About 21% (9 participants) reported no pain in the last 12 months. For those experiencing pain, 7.1% (3 participants) rated their pain severity between 1 to 3, indicating mild pain. The majority, 48% (20 participants), reported severe pain severity, rated between 7 to 9, while 24% (10 participants) indicated a pain severity level between 4 to 6, representing moderate pain.

Table 3 : Description of Acute Painful Events.

Characteristic	N[1]= 42
Pain Frequency - Categorised (In 12 Months)	
Very High(>12 Acute Pain Crisis)	2 (4.8%)
High(7-12 Acute Pain Crisis)	5 (12%)
Moderate(3-6 Acute Pain Crisis)	15 (36%)
Low(0-2 Acute Pain Crisis)	20 (48%)
Pain Duration (Days)	4.00 (2.00, 5.75)
Pain Duration - Categorised (Days)	
Very Long (>10)	1 (2.4%)
Long (7-10)	8 (19.4%)
Moderate (4-6)	15 (36%)
Short (0-3)	18 (43%)
Pain Severity (By Wong-Baker Faces Pain Rating Scale)	
0	9 (21%)
1 To 3	3 (7.1%)
4 To 6	10 (24%)
7 To 9	20 (48%)

[1]Frequency ; n%

Over the past 12 months, the study population utilized various healthcare facilities, with the majority (52%) identifying tertiary care as their primary and most frequent healthcare contact. Private clinics were the second most common facility, used by 29% of participants, while private hospitals accounted for 9.5% of primary contacts. Primary and secondary care facilities were the least used, each representing 4.8% of participants. In terms of hospitalization frequency, most participants had either two visits (33%) or one visit (17%). A smaller group had four visits (19%), followed by 9.5% who had three or six visits. Higher visit frequencies were observed in a few cases, with 2.4% reporting eight, twelve, or twenty visits over the year. When categorized, 55% fell into

the low-frequency visit group (0-2 visits), while 40.4% had high-frequency visits (3-10), and 4.8% experienced very high-frequency visits (>10).

The main reason for healthcare visits was pain, accounting for 60% of visits, followed by weakness (24%) and fever (7.1%). Other reasons for healthcare utilization made up 9.5% of cases. Regarding blood transfusion frequency, 62% of participants did not receive any transfusions in the past year, while 12% had one transfusion and 14% had two transfusions. Smaller groups received more frequent transfusions, with 2.4% receiving three, 7.1% receiving four, and 2.4% receiving eight transfusions. In terms of the volume of PRBC transfused, 62% did not require any transfusions, while 9.5% received two units and 7.1% received four units. Additional participants received varying amounts, including 4.8% who received three units, 9.5% who received six units, and 2.4% who required twelve units.

Table 4 : Description of Hospitalization Events.

Characteristic	N[1] = 42
Hospitalisation (First And Most Frequent Contact)	
Private Clinic	12 (29%)
Private Hospital	4 (9.5%)
Primary	2 (4.8%)
Secondary	2 (4.8%)
Tertiary	22 (52%)
Frequency Of Hospitalisation Categorised (12 Months)	
Very High(>10)	2 (4.8%)
High(3-10)	16 (40.4%)
Low(0-2)	23 (55%)
Reason For Hospitalisation	
Fever	3 (7.1%)
Pain	25 (60%)
Weakness	10 (24%)
Others (Leg Ulcers, CCF, Etc)	4 (9.5%)

[1]Frequency ; n%

Table 5 : Description of Transfusion requirements .

Characteristic	N[1] = 42
Blood Transfusion Frequency In Last 12 Months	
0	26 (62%)
1	5 (12%)
2	6 (14%)
3	1 (2.4%)
4	3 (7.1%)
8	1 (2.4%)
Units Of PRBC Transfusion In The Last 12 Months	
0	26 (62%)
2	4 (9.5%)
3	2 (4.8%)
4	3 (7.1%)
5	2 (4.8%)
6	4 (9.5%)
12	1 (2.4%)

[1]Frequency ; n%

Table 6 : Description of Health Education frequency.

Characteristic	N[1] = 42
Health Education	
Never	5 (12%)
Occasional	18 (43%)
Regular	19 (45%)

[1]Frequency ; n%

Table 7 : Description of Medication history.

Characteristic	N[1] = 42
Taking Hydroxyurea	
No	6 (14%)
Yes	36 (86%)
Taking Folic Acid	
N (Fa)	7 (17%)
Y(Fa)	35 (83%)
Hydroxyurea Dose	N[1] = 36
High Dose	17 (47%)
Low Dose	19 (53%)
Medication Compliance	
Non Compliant	16 (44%)
Compliant	20 (56%)

[1]Frequency ; n%

The medication history and health awareness data of the study participants present insights into the use of hydroxyurea and folic acid, along with compliance patterns, dosing regimens, and levels of health education.

Among the 42 participants, hydroxyurea usage was prevalent, with 86% (36 participants) reporting that they use the medication, while only 14% (6 participants) did not. Folic acid (FA) supplementation was also common, with 83% (35 participants) taking FA as part of their treatment regimen, leaving 17% (7 participants) who were not using it. This indicates that the majority of participants were on a combination of hydroxyurea and folic acid, which are often used together in sickle cell anemia management to reduce the frequency of pain crises and support red blood cell health.

Health education was provided at varying levels. Regular health education was reported by 45% (19 participants), indicating consistent exposure to disease-related information. Occasional health education was noted by 43% (18 participants). A smaller group, 12% (5 participants), had never received health education, highlighting a gap in patient awareness and disease management knowledge for some individuals.

Among the 36 participants using hydroxyurea, dosing and compliance data were further analyzed. In terms of dosage, 53% (19 participants) were on a low-dose regimen, while 47% (17 participants) received a high dose. For compliance, 56% (20 participants) of those taking hydroxyurea reported adherence to their medication regimen, while 44% (16 participants) indicated non-compliance. This data suggests that although a majority were compliant, a considerable portion of participants experienced challenges in maintaining regular use of hydroxyurea, which may impact the effectiveness of their treatment.

According to Table 8; the median hemoglobin (Hb) level among the participants was 8.20 g/dL, with an interquartile range (IQR) of 7.03 to 10.05 g/dL, indicating generally low Hb levels typical of individuals with sickle cell anemia. Total Leukocyte Count (TLC) was relatively high, with a median value of 10.1 $x10^9$/L (IQR: 7.1 to 16.5 $x10^9$/L), suggesting a heightened immune response, possibly due to chronic inflammation or infection. Platelet count (PLT) had a median value of 243 $x10^9$/L, with an IQR from 160 to 397 $x10^9$/L, demonstrating variability in platelet levels among participants. In terms of differential counts, the percentage of neutrophils (P%) had a median of 58% (IQR: 49-69%), while lymphocyte percentage (L%) had a median of 30% (IQR: 22-41%), reflecting typical values within the context of chronic disease. Reticulocyte count (RETIC), which is an indicator of red blood cell production, had a notably high median of 7.8% (IQR: 6.0-9.6%), reflecting increased erythropoietic activity as the body compensates for hemolysis.

Table 8 : Description of Hematological and Biochemical parameters.([1]Frequency ; n%*)*

Parameters	N[1] = 42
Hb	8.20 (7.03, 10.05)
TLC	10.1 (7.1, 16.5)
PLT	243 (160, 397)
P%	58 (49, 69)
L%	30 (22, 41)
RETIC	7.8 (6.0, 9.6)
HBS	70 (63, 75)
HBA0	5 (3, 7)
HBA2	2.80 (1.63, 3.58)
HBF	16 (11, 23)
HPLC Interpretation	
AS	2 (4.8%)
SB+	17 (40%)
SB0	3 (7.1%)
SS	20 (48%)
HbF Interpretation	
High(>20)	15 (36%)
Low(<20)	27 (64%)
D DIMER	2.00 (1.18, 2.74)
UREA	21 (13, 26)
CREAT	0.64 (0.57, 0.75)
UACR	
MACROALBUMINURIA	4 (9.5%)
MICROALBUMINURIA	3 (7.1%)
NO ALBUMINURIA	35 (83%)

Table 9 : Cardiac biomarkers distribution.

Parameters	N[1] = 42
BNP	
10(BELOW DETECTION RANGE)	36 (86%)
26	1 (2.4%)
88	1 (2.4%)
255	1 (2.4%)
368	1 (2.4%)
1024	1 (2.4%)
1595	1 (2.4%)

[1]Frequency ; n%

Hemoglobin electrophoresis (HPLC) patterns showed that 48% (20 participants) had the SS genotype, indicating homozygous sickle cell disease. The SB+ genotype was present in 40% (17 participants), SB0 in 7.1% (3 participants), and AS genotype in 4.8% (2 participants), reflecting a mix of sickle cell variants. Based on fetal hemoglobin (HbF) levels, 36% (15 participants) had high HbF (>20%), while 64% (27 participants) had lower HbF levels (<20%).

The median D-dimer level was 2.00 µg/mL (IQR: 1.18-2.74 µg/mL), which may indicate a tendency toward hypercoagulability and risk of thrombotic events. Urea levels had a median of 21 mg/dL (IQR: 13-26 mg/dL), while creatinine levels were relatively low with a median of 0.64 mg/dL (IQR: 0.57-0.75 mg/dL), suggesting generally preserved renal function in most participants. Microalbuminuria was assessed through urinary albumin excretion, with a median value of 11 mg/g (IQR: 2-23 mg/g). For urine albumin-to-creatinine ratio (UACR), 83% (35 participants) exhibited no albuminuria, indicating normal kidney function, while 7.1% (3 participants) had microalbuminuria, and 9.5% (4 participants) presented with macroalbuminuria, suggesting early-stage renal impairment in a small subset.

Brain natriuretic peptide (BNP), an indicator of cardiac stress, was generally low for most participants, with 86% (36 participants) falling below the detection threshold. However, a few participants had elevated BNP levels, with individual readings of 26, 88, 255, 368, 1024, and 1595 pg/mL, each representing 2.4% (1 participant) of the population. These elevated levels suggest the presence of cardiac stress or strain in a small subset of the study group, which may be associated with complications like pulmonary hypertension and/or heart failure.

For assessment of long term complications we used imaging parameters such as 2D echocardiography and FICAT and ARLET classification, which is a comprehensive multimodal score for classifying stages of Avascular Necrosis of Femoral Hip. The Ficat and Arlet classification system, which assesses avascular necrosis based on X-ray ,MRI findings and Bone Scan, showed that a large majority of participants (74%, 31 individuals) were classified as Stage 0, indicating no evidence of avascular necrosis. This suggests that, for most participants, there were no significant musculoskeletal complications visible through imaging. Stages 2 and 3 each accounted for 12% (5 participants each), reflecting moderate to advanced stages of avascular necrosis, while a small percentage (2.4%, 1 participant) reached Stage 4, indicating severe necrosis with likely joint damage or collapse. Notably, no participants were classified at Stage 1, which may imply a progression directly from asymptomatic stages to more advanced stages for those affected.

Table 10 : Imaging parameters of the participants - FICAT and ARLET Classification

Characteristic	N[1] = 42
FICAT and ARLET Classification	
0	31 (74%)
1	0 (0%)
2	5 (12%)
3	5 (12%)
4	1 (2.4%)

[1]Frequency ; n%

Table 11 : Imaging parameters of the participants - 2D Echocardiography.

Characteristic	N[1] = 42
Systolic Dysfunction	
Normal	39 (93%)
Mild Dysfunction (SYS 1)	1 (2.4%)
Moderate Dysfunction (SYS 2)	2 (4.8%)
Severe Dysfunction (SYS 3)	0 (0%)
Diastolic Dysfunction	
Normal	36 (86%)
Mild Dysfunction (DYS 1)	6 (14%)
Moderate Dysfunction (DYS 2)	0 (0%)
Severe Dysfunction (DYS 3)	0 (0%)

[1]Frequency ; n%

Systolic Dysfunction

Cardiac imaging for systolic dysfunction indicated that most participants (93%, 39 individuals) exhibited normal systolic function, suggesting preserved left ventricular ejection capabilities. Only a small subset showed signs of systolic impairment, with 2.4% (1 participant) categorized as SYS 1, representing mild systolic dysfunction, and 4.8% (2 participants) classified as SYS 2, indicating moderate systolic dysfunction. No cases of severe systolic dysfunction (SYS 3) were reported in this study group, which suggests that, for most participants, systolic cardiac function is maintained, possibly reflecting an absence of advanced cardiac complications.

Diastolic Dysfunction

In terms of diastolic dysfunction, 86% (36 participants) demonstrated normal diastolic function, indicating adequate ventricular relaxation and filling during diastole. A

portion of participants, however, did exhibit mild diastolic dysfunction, with 14% (6 participants) classified as DYS 1. There were no cases of moderate (DYS 2) or severe (DYS 3) diastolic dysfunction, suggesting that while some participants experience mild issues with ventricular filling, more advanced diastolic impairment is not common within this group.

The hematological parameters in patients with sickle cell disease, categorized by the frequency of acute pain crises into low (0-2 episodes), moderate (3-6 episodes), high (7-12 episodes), and very high (>12 episodes) groups. The data includes median values and interquartile ranges (IQR) for various blood markers such as hemoglobin, total leukocyte count, platelet count, and others, analyzed using the Kruskal-Wallis rank sum test and Fisher's exact test to explore potential differences across these groups.

A small subgroup of patients (N=15) underwent pulmonary function testing (PFT). Among these, 80% (12 patients) displayed no abnormalities, while the remaining patients showed a restrictive flow pattern. Due to operational limitations, only one patient could undergo a Tc99 triple-phase bone scan. This scan revealed signs of avascular necrosis (AVN) in both femoral heads, accompanied by patchy tracer uptake and heterogeneous sclerosis in the proximal humeri, sternum, medial end of the lateral clavicle, cervico-dorso-lumbar vertebrae, sacrum, and bilateral pelvic bones. It also showed area of dystrophic calcification in a contracted spleen. The patient, a 41-year-old with HbSS and low HbF levels, experienced moderate pain episodes (3-6 per year), typically short in duration (0-3 days), with a high frequency of hospitalizations (3-10 admissions), primarily for pain management. They adhered to a low-dose hydroxyurea regimen and received regular health education, with no other organ dysfunctions noted. Due to the limited number of patients undergoing PFT and bone scans, these data were not included in the overall analysis.

Few patients (1-2 participants) also had developed other complications of Sickle Cell Disease, such as Hemochromatosis, Pulmonary Hypertension, Non Healing Leg Ulcers, etc. Due to such small numbers these complications were not considered in final analysis.

Figure 5 : Triple phase Bone Scan Image 1 of Patient P.J.

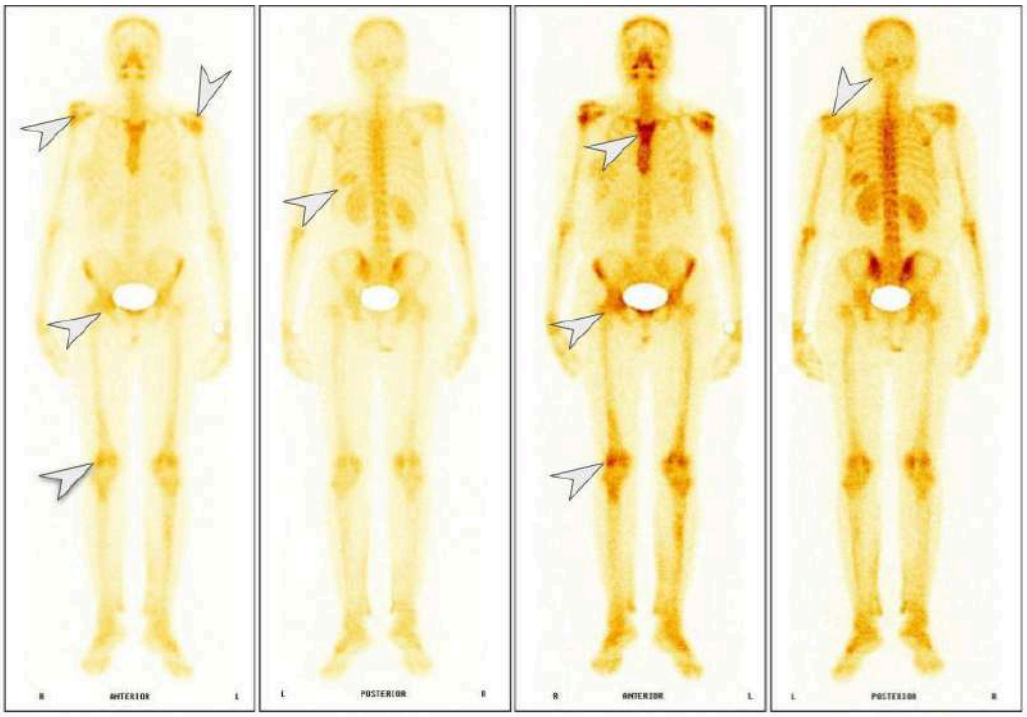


Figure shows Area of patchy tracer uptake in bilateral femoral head, bilateral proximal humerus head, sternum, medial end of bilateral clavicle, cervico-dorso-lumbar vertebrae with sacrum, bilateral pelvis , bilateral proximal tibia.

Figure 6 : Triple phase Bone Scan Image 2 of Patient P.J.

Figure shows SPECT - CT images showing increased uptake over Sternum and Vertebrae.

Figure 7 : Triple phase Bone Scan Image 3 of Patient P.J.

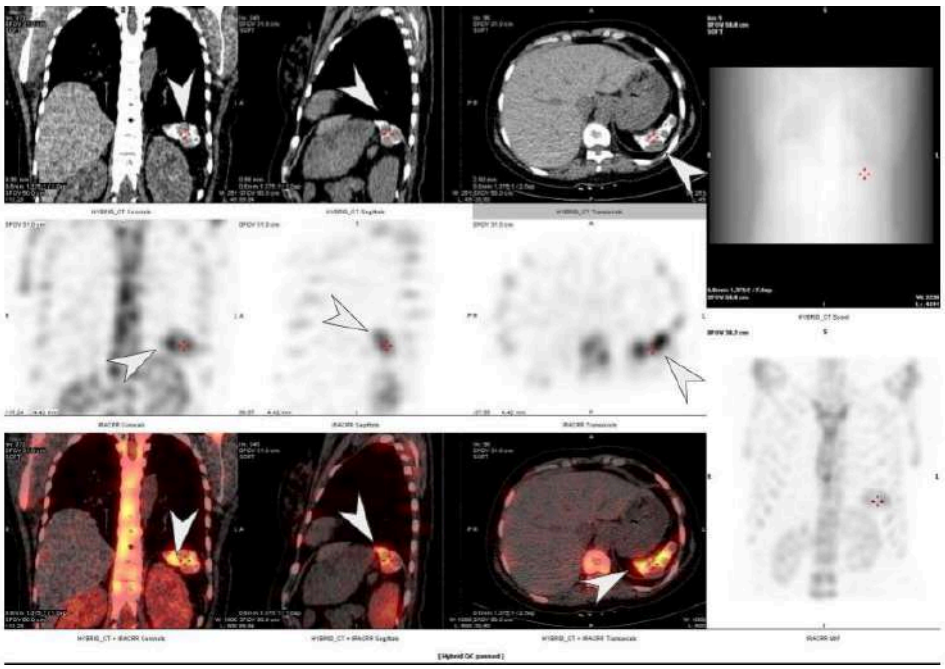


Image shows moderately increased tracer uptake in patchy calcification of small contracted spleen suggestive of dystrophic calcification.

Figure 8 : Bone Scan Image 4 of Patient P.J.

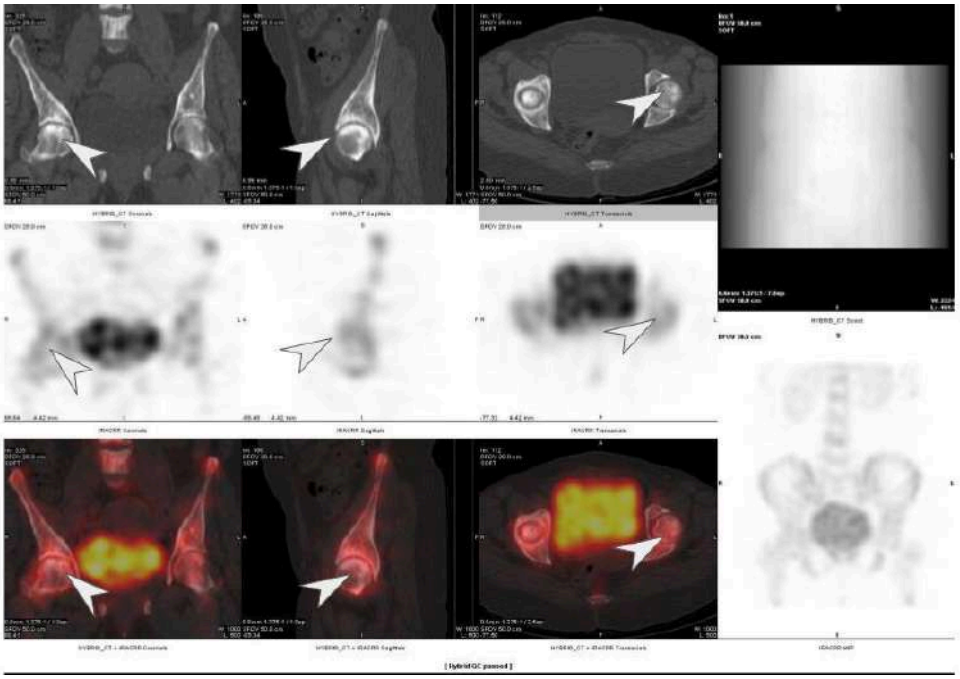


Image shows areas of patchy increased tracer uptake with areas of sclerosis in head of bilateral femur seen in SPECT- CT

Figure 9 : Bone Scan Image 5 of Patient P.J.

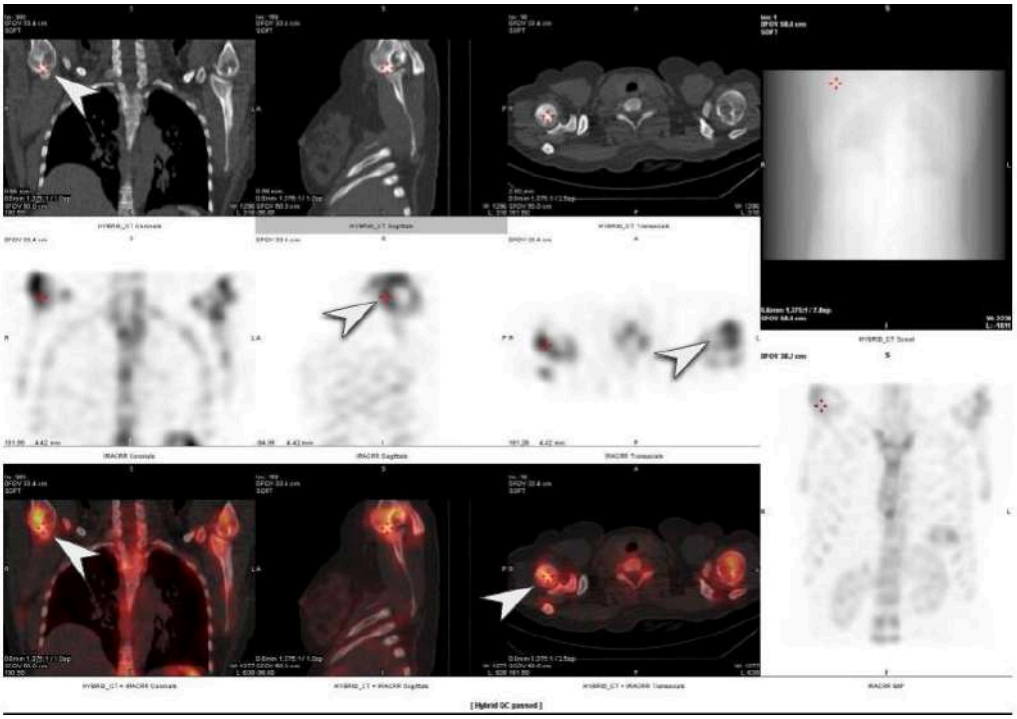


Image shows areas of patchy increased tracer uptake with areas of sclerosis in head of bilateral humerus seen in SPECT- CT

Table 12 : Correlating hematological parameters with frequency of acute painful crises.

Characteristic	LOW(0-2), N = 20[1]	MODERATE(3-6), N = 15[1]	HIGH(7-12), N = 5[1]	VERY HIGH(>12), N = 2[1]	p-value[2]
Hb	8.90 (7.88, 10.20)	7.80 (6.80, 9.95)	8.70 (7.50, 8.90)	7.40 (7.30, 7.50)	0.5
TLC	7.9 (6.5, 14.5)	16.5 (10.3, 19.0)	8.1 (4.3, 9.4)	11.5 (10.3, 12.8)	0.056
PLT	239 (172, 354)	389 (195, 437)	135 (110, 171)	438 (380, 497)	0.10
HbHPLC interpretation					0.8
AS	1 (5.0%)	0 (0%)	1 (20%)	0 (0%)	
SB+	9 (45%)	5 (33%)	2 (40%)	1 (50%)	
SB0	2 (10%)	1 (6.7%)	0 (0%)	0 (0%)	
SS	8 (40%)	9 (60%)	2 (40%)	1 (50%)	
HbF	15 (13, 24)	16 (10, 21)	23 (18, 26)	10 (10, 10)	0.4
HbS	68 (61, 75)	72 (68, 79)	63 (40, 70)	77 (74, 81)	0.062
Reticulocyte index	6.9 (4.9, 8.6)	8.8 (7.3, 10.5)	6.1 (3.9, 8.8)	7.3 (6.9, 7.7)	0.3
D dimer	2.05 (1.12, 2.82)	2.41 (1.31, 2.84)	0.55 (0.34, 1.22)	2.10 (2.05, 2.15)	0.061

[1] Median (IQR); n (%)

[2] Kruskal-Wallis rank sum test; Fisher's exact test

Table 12 examines the relationship between hematological parameters and the frequency of acute pain crises among sickle cell disease patients, categorized into 'low' (0-2 episodes), 'moderate' (3-6 episodes), 'high' (7-12 episodes), and 'very high' (>12

episodes) groups. Hemoglobin (Hb) levels decrease with rising pain frequency, ranging from a median of 8.90 g/dL in the 'low' group to 7.40 g/dL in the 'very high' group, although this is not statistically significant ($p = 0.5$). Total Leukocyte Count (TLC) and D-dimer levels, which reflect inflammation and coagulation, are highest in the 'moderate' group with p-values close to significance (TLC: $p = 0.056$, D-dimer: $p = 0.061$). Similarly, platelet count (PLT) and sickle hemoglobin (HbS) levels increase with pain frequency, with the 'very high' group showing the highest medians at 438 $x10^9$/L for PLT and 77% for HbS, though without statistical significance (PLT: $p = 0.10$, HbS: $p = 0.062$).

Table 13 : Correlating hematological parameters with frequency of Hospitalisation.

Characteristic	LOW(0-2), N = 231	HIGH(3-10), N = 171	VERY HIGH(>10), N = 21	p-value2
Hb	8.30 (6.55, 10.10)	8.10 (7.50, 9.80)	8.05 (7.63, 8.48)	>0.9
TLC	8.2 (6.1, 15.8)	10.9 (8.1, 16.5)	12.3 (11.4, 13.2)	0.6
PLT	241 (167, 396)	245 (135, 389)	454 (403, 504)	0.3
HbHPLC interpretation				0.8
AS	1 (4.3%)	1 (5.9%)	0 (0%)	
SB+	9 (39%)	6 (35%)	2 (100%)	
SB0	2 (8.7%)	1 (5.9%)	0 (0%)	
SS	11 (48%)	9 (53%)	0 (0%)	
HbF	15 (12, 22)	19 (11, 23)	14 (12, 16)	0.7
HbS	70 (63, 75)	70 (63, 77)	71 (71, 71)	>0.9
Reticulocyte index	7.5 (5.7, 9.5)	6.5 (6.0, 8.8)	9.5 (8.8, 10.2)	0.7
D dimer	2.10 (1.07, 2.84)	1.62 (1.22, 2.47)	1.17 (0.76, 1.59)	0.4

[1] Median (IQR); n (%)

[2] Kruskal-Wallis rank sum test; Fisher's exact test

Table 13 evaluates the relationship between hematological parameters and hospitalization frequency in sickle cell disease patients, divided into 'low' (0-2), 'high' (3-10), and 'very high' (>10) hospitalization groups. The analysis used the Kruskal-Wallis rank sum test for continuous variables and Fisher's exact test for categorical variables, but no statistically significant correlations were found across the groups.

Hemoglobin (Hb) levels were similar across hospitalization groups, with medians ranging from 8.05 to 8.30 g/dL ($p > 0.9$). Total leukocyte count (TLC) showed a non-significant increase in the 'very high' hospitalization group, with a median of 12.3 $x10^9$/L ($p = 0.6$). Platelet counts (PLT) were highest in the 'very high' group, with a median of 454 $x10^9$/L, suggesting a trend toward increased coagulation potential, although this was not statistically significant ($p = 0.3$).

Regarding hemoglobin variant distribution (HbHPLC interpretation), the SB+ genotype was present in 100% of the 'very high' hospitalization group, while the SS genotype predominated in the 'low' and 'high' groups; however, this difference was not significant ($p = 0.8$). Fetal hemoglobin (HbF) levels were slightly lower in the 'very high' group, with a median of 14%, compared to 19% in the 'high' group and 15% in the 'low' group ($p = 0.7$). Sickle hemoglobin (HbS) levels were consistent across groups ($p > 0.9$).

The reticulocyte index was elevated in the 'very high' group, suggesting increased erythropoietic activity, though this was also not statistically significant ($p = 0.7$). D-dimer levels, which reflect coagulation activity, were highest in the 'low' hospitalization group at 2.10 µg/mL, with no significant differences observed ($p = 0.4$). Overall, while certain trends were noted, none of the hematological parameters demonstrated statistically significant correlations with hospitalization frequency in this analysis.

Table 14 : Correlating FICAT and ALRET staging with Acute Pain crisis and Hospitalization events.

Characteristic	0, N = 31[1]	1, N = 0[1]	2, N = 5[1]	3, N = 5[1]	4, N = 1[1]	p-value [2]
Acute Painful Crisis in 12 months (Categorised)						0.10
HIGH(7-12)	1 (3.2%)	0 (0%)	2 (40%)	2 (40%)	0 (0%)	
LOW(0-2)	17 (55%)	0 (0%)	1 (20%)	1 (20%)	1 (100%)	
MODERATE(3-6)	11 (35%)	0 (0%)	2 (40%)	2 (40%)	0 (0%)	
VERY HIGH(>12)	2 (6.5%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
Pain duration in days	4.00 (1.00, 5.00)	0 (0%)	4.00 (3.00, 4.00)	7.00 (4.00, 7.00)	10.00 (10.00, 10.00)	0.12
Hospitalization frequency in 12 months (categorized)						**0.015**
LOW (0-2)	21 (68%)	0 (0%)	1 (20%)	0 (0%)	1 (100%)	
HIGH (3-10)	9 (29.2%)	0 (0%)	4 (80%)	4 (80%)	0 (0%)	
VERY HIGH (>10)	1 (3.2%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	

[1] n (%); Median (IQR)

[2] Fisher's exact test; Kruskal-Wallis rank sum test.

Table 15 : Correlating FICAT and ALRET staging with Blood Transfusion frequency.

Characteristic	0, N = 31[1]	1, N = 0[1]	2, N = 5[1]	3, N = 5[1]	4, N = 1[1]	p-value[2]
Blood Transfusion Frequency in 12 months						0.5
0	20 (65%)	0 (0%)	2 (40%)	3 (60%)	1 (100%)	
1	4 (13%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	
2	3 (9.7%)	0 (0%)	3 (60%)	0 (0%)	0 (0%)	
3	1 (3.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
4	2 (6.5%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)	
8	1 (3.2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	

[1] n (%); Median (IQR)

[2] Fisher's exact test; Kruskal-Wallis rank sum test.

The correlation between FICAT staging (0, 1, 2, 3, and 4) and the frequency of acute pain crises, healthcare utilization, and blood transfusion needs among patients with sickle cell disease is shown in Table 14 and Table 15. Notably, a statistically significant difference was observed in the frequency of hospitalizations ($p = 0.015$) across FICAT stages. Patients in FICAT stages 2 and 3 showed higher healthcare utilization, with 80% of these participants making between 3 to 10 visits annually, compared to 29.2% in FICAT 0. While trends indicated more frequent and prolonged pain episodes in advanced FICAT stages, as well as increased blood transfusion needs, these differences were not statistically significant.

Table 16 : Correlating FICAT & ALRET staging with Hematological parameters

Characteristic	0, N = 31[1]	1, N = 0[1]	2, N = 5[1]	3, N = 5[1]	4, N = 1[1]	p-value[2]
Hb	7.90 (6.80, 10.00)	0(0,0)	9.10 (8.10, 10.30)	8.90 (8.70, 9.80)	7.90 (7.90, 7.90)	0.5
TLC	9.0 (6.9, 17.0)	0(0,0)	9.4 (7.1, 10.9)	10.5 (9.7, 14.3)	11.6 (11.6, 11.6)	0.9
PLT	245 (174, 396)	0(0,0)	113 (110, 207)	420 (352, 454)	241 (241, 241)	0.14
HbHPLC Interpretation						0.4
AS	1 (3.2%)	0(0%)	1 (20%)	0 (0%)	0 (0%)	
SB+	14 (45%)	0(0%)	2 (40%)	1 (20%)	0 (0%)	
SB0	2 (6.5%)	0(0%)	1 (20%)	0 (0%)	0 (0%)	
SS	14 (45%)	0(0%)	1 (20%)	4 (80%)	1 (100%)	
HbF	14 (11, 21)	0(0%)	21 (9, 22)	19 (18, 23)	24 (24, 24)	0.4
HbS	**72 (65, 78)**	**0(0,0)**	**46 (40, 65)**	**71 (70, 72)**	**69 (69, 69)**	**0.048**
Reticulocyte Index	7.5 (6.0, 9.0)	0(0,0)	9.8 (8.8, 10.5)	6.1 (3.4, 8.7)	7.3 (7.3, 7.3)	0.7
D dimer	2.10 (1.24, 2.84)	0(0,0)	2.41 (1.62, 2.59)	1.22 (0.59, 1.34)	0.97 (0.97, 0.97)	0.13

[1] Median (IQR); n (%)

[2] Kruskal-Wallis rank sum test; Fisher's exact test

Table 16 correlates FICAT staging with various hematological parameters among patients with sickle cell disease. A statistically significant difference was observed in **Hemoglobin S (HbS)** levels across the FICAT stages ($p = 0.048$). Patients in FICAT stage 2 had a lower median HbS of 46% (IQR: 40-65), while those in stages 0, 3, and 4 had higher medians, with FICAT 0 at 72% (IQR: 65-78), FICAT 3 at 71% (IQR: 70-72), and FICAT 4 at 69%. This suggests a potential relationship between increasing FICAT stage and varying HbS levels, with stage 2 patients showing notably lower sickle hemoglobin concentrations. Other parameters, including hemoglobin, total leukocyte count, platelet count, HbF, reticulocyte index, and D-dimer, varied across the stages but did not show statistically significant differences.

Table 17 : Correlation of Systolic function (2D ECHO) with Hematological parameters.

Characteristic	Normal, N = 39[1]	Mild Systolic Dysfunction, N = 1[1]	Mod. Systolic Dysfunction, N = 2[1]	Severe Systolic Dysfunction, N = 0[1]	p-value[2]
Hb	**8.60 (7.35, 10.10)**	**5.30 (5.30, 5.30)**	**5.55 (5.33, 5.78)**	**0(0, 0)**	**0.042**
TLC	10.5 (6.9, 16.5)	42.4 (42.4, 42.4)	7.9 (7.8, 8.0)	0(0, 0)	0.2
PLT	278 (174, 408)	99 (99, 99)	180 (167, 193)	0(0, 0)	0.15
HbHPLC Interpretation					0.2
AS	2 (5.1%)	0 (0%)	0 (0%)	0 (0%)	
SB+	15 (38%)	1 (100%)	1 (50%)	0(0, 0)	
SB0	2 (5.1%)	0 (0%)	1 (50%)	0(0, 0)	
SS	20 (51%)	0 (0%)	0 (0%)	0 (0%)	
HbF	16 (11, 23)	19 (19, 19)	13 (13, 14)	0(0, 0)	0.8
HbS	70 (64, 75)	69 (69, 69)	63 (55, 71)	0(0, 0)	>0.9
Reticulocyte Index	7.5 (6.0, 9.5)	12.3 (12.3, 12.3)	5.7 (4.5, 6.9)	0(0, 0)	0.3
D dimer	1.99 (1.07, 2.60)	2.90 (2.90, 2.90)	2.44 (2.17, 2.71)	0(0, 0)	0.2

[1] n (%); Median (IQR)

[2] Fisher's exact test; Kruskal-Wallis rank sum test

Table 17 compares hematological parameters across different levels of systolic function (normal, mild, moderate, and severe) as assessed by 2D echocardiography (2D ECHO) in patients with sickle cell disease. Notably, **hemoglobin (Hb)**levels showed a statistically significant difference across groups ($p = 0.042$). Patients with normal systolic function had a median Hb of 8.60 g/dL (IQR: 7.35-10.10), while those with mild and moderate systolic dysfunction had substantially lower Hb levels, with medians of 5.30 g/dL and 5.55 g/dL, respectively. This suggests a potential association between lower Hb levels and the presence of systolic dysfunction.

Other parameters, including total leukocyte count (TLC), platelet count (PLT), HbF, HbS, reticulocyte index, and D-dimer, showed trends of variation across different levels of systolic function, though they did not reach statistical significance. For instance, TLC was markedly elevated in the mild dysfunction group at 42.4 $x10^9$/L, and D-dimer levels were highest in this group as well at 2.90 µg/mL, indicating possible inflammation and coagulation activity. However, the significant finding remains the reduced Hb levels in those with systolic dysfunction, highlighting the potential impact of anemia severity on cardiac function in this population.

Table 18 : Correlation of Diastolic function (2D ECHO) with Hematological parameters.

Characteristic	Normal, N = 36[1]	Mild diastolic dysfunction, N = 6[1]	Mod. diastolic dysfunction, N = 0[1]	Severe diastolic dysfunction, N = 0[1]	p-value[2]
Hb	8.45 (7.43, 9.95)	6.35 (5.48, 9.55)	0 (0, 0)	0 (0, 0)	0.3
TLC	11.1 (7.1, 16.6)	7.9 (7.3, 8.2)	0 (0, 0)	0 (0, 0)	0.5
PLT	262 (176, 418)	180 (113, 307)	0 (0, 0)	0 (0, 0)	0.12
HbHPLC Interpretation					0.031
AS	2 (5.6%)	0 (0%)	0 (0%)	0 (0%)	
SB+	12 (33%)	5 (83%)	0 (0%)	0 (0%)	
SB0	2 (5.6%)	1 (17%)	0 (0%)	0 (0%)	
SS	20 (56%)	0 (0%)	0 (0%)	0 (0%)	
HbF	17 (10, 24)	15 (14, 18)	0 (0, 0)	0 (0, 0)	0.9
HbS	70 (62, 76)	68 (65, 74)	0 (0, 0)	0 (0, 0)	0.7
Reticulocyte Index	7.4 (5.8, 9.3)	8.2 (7.5, 9.9)	0 (0, 0)	0 (0, 0)	0.6
D dimer	1.92 (0.93, 2.50)	2.83 (2.12, 2.90)	0 (0, 0)	0 (0, 0)	0.091

[1] n (%); Median (IQR)

[2] Fisher's exact test; Wilcoxon rank sum test

The patients are categorized into normal, mild, moderate, and severe diastolic dysfunction groups. The only statistically significant finding is in the HbHPLC interpretation across these groups (p = 0.031). The SB+ genotype was observed in 33% of patients with normal diastolic function and increased to 83% in those with mild

diastolic dysfunction. Meanwhile, the SS genotype was predominant (56%) in the normal group but absent in the mild diastolic dysfunction group. This suggests a potential association between the Hb genotype and the likelihood of developing mild diastolic dysfunction, particularly with an increased presence of the SB+ genotype in affected individuals.

Other parameters, such as hemoglobin (Hb), total leukocyte count (TLC), platelet count (PLT), HbF, HbS, reticulocyte index, and D-dimer, showed differences between the groups but did not reach statistical significance. For example, the median D-dimer level was higher in patients with mild diastolic dysfunction (2.83 µg/mL) compared to those with normal function (1.92 µg/mL), suggesting a trend towards increased coagulation activity in diastolic dysfunction. However, the p-value of 0.091 indicates this was not statistically significant.

Table 19 : Correlation of Microalbuminuria with Hematological parameters

Characteristic	No Albuminuria, N = 35[1]	Microalbuminuria, N = 3[1]	Macroalbuminuria, N = 4[1]	p-value[2]
Hb	8.60 (7.35, 10.00)	5.30 (5.15, 8.10)	6.90 (5.78, 8.48)	0.3
TLC	10.9 (6.9, 16.5)	8.3 (6.6, 25.3)	8.2 (8.0, 12.3)	>0.9
PLT	245 (174, 395)	113 (106, 299)	300 (193, 400)	0.7
HbHPLC Interpretation				0.7
AS	2 (5.7%)	0 (0%)	0 (0%)	
SB+	14 (40%)	1 (33%)	2 (50%)	
SB0	2 (5.7%)	0 (0%)	1 (25%)	
SS	17 (49%)	2 (67%)	1 (25%)	
HbF	15 (10, 23)	21 (20, 34)	15 (14, 16)	0.3
HbS	70 (64, 75)	69 (63, 70)	73 (62, 79)	0.8
Reticulocyte Index	7.5 (6.0, 9.5)	12.3 (10.3, 13.7)	5.4 (3.4, 7.5)	0.079
D dimer	2.10 (1.09, 2.69)	1.84 (1.23, 2.37)	1.62 (1.30, 2.17)	>0.9

[1] Median (IQR); n (%)

[2] Kruskal-Wallis rank sum test; Fisher's exact test

Table 19 explores the relationship between albuminuria levels (no albuminuria, microalbuminuria, and macroalbuminuria) and various hematological parameters among patients with sickle cell disease. While no statistically significant differences were observed across albuminuria groups, some trends are notable. Hemoglobin (Hb) levels were highest in patients without albuminuria, with a median of 8.60 g/dL, compared to lower levels in the microalbuminuria and macroalbuminuria groups, with medians of 5.30 g/dL and 6.90 g/dL, respectively (p = 0.3). Total Leukocyte Count (TLC) and Platelet Count (PLT) did not show significant variation across the groups, though the

platelet count was somewhat higher in the macroalbuminuria group (median 300 $x10^9$/L) compared to the other groups.

The distribution of hemoglobin genotypes (HbHPLC interpretation) was similar across albuminuria levels, with the SS genotype present in all groups and most common in the no albuminuria group (49%) and less frequent in the macroalbuminuria group (25%). Fetal hemoglobin (HbF) levels were slightly higher in the microalbuminuria group (median 21%) compared to the no albuminuria and macroalbuminuria groups. Reticulocyte index showed a trend towards significance ($p = 0.079$), with elevated levels in the microalbuminuria group (median 12.3%) indicating increased erythropoiesis. D-dimer levels, which reflect coagulation activity, showed no significant differences across albuminuria levels, with a median of 2.10 µg/mL in the no albuminuria group.

Discussion

Sickle cell disease (SCD), a genetically inherited blood disorder, affects millions worldwide, with significant morbidity due to its complications, including acute pain crises, chronic anemia, organ damage, and a variety of systemic impacts. The study population was primarily from Madhya Pradesh, India, representing a demographic with notable SCD prevalence, particularly within tribal populations where the sickle cell trait can range from 40% to 55% in some areas (**Raman** et al., 2021) . This distribution underscores the need for targeted health interventions to address SCD in these regions. The participants had a median age of 25 years and were predominantly male, with a notable percentage of individuals living above the poverty line, suggesting a mix of socioeconomic backgrounds.

Acute Pain Events and Hospitalizations

Acute pain crises were reported with varying frequencies, with nearly half of the participants experiencing low pain frequency (0-2 episodes per year). This aligns with findings from **Rees** et al. (2016) that emphasize the episodic nature of pain in SCD due to vaso-occlusion . The association between pain frequency and hematological markers, such as elevated reticulocyte indices and platelet counts, suggests an ongoing erythropoietic response to hemolysis, as supported by **Tsaras** et al., who reported that the reticulocyte count correlates with disease severity in SCD . Additionally, although not statistically significant, the trend towards higher total leukocyte count (TLC) and D-dimer levels among individuals with moderate pain frequency could reflect an inflammatory state, consistent with **Naik Haywood** et al.'s research on the inflammatory cascade in SCD .

Correlation of Hematological Parameters with Hospitalization Frequency

The correlation analysis did not reveal statistically significant differences in hematological parameters across different hospitalization frequency groups, although some trends were observed. Individuals with very high hospitalization frequency showed elevated median TLC, platelet counts, and reticulocyte indices. Elevated TLC is often associated with an inflammatory response, as observed by **Naik Haywood** et al. ,

while increased platelet counts and reticulocyte indices can indicate a higher erythropoietic drive in response to anemia (**Tsaras** et al.). This study's lack of statistically significant findings across hospitalization groups could be attributed to the limited sample size but provides an important observational insight into potential markers that could predict disease severity in larger populations.

Imaging Parameters: Cardiopulmonary and Musculoskeletal Implications

Echocardiography identified some degree of cardiac dysfunction, with mild diastolic dysfunction noted in a small subset of patients. This finding is consistent with **Lande** et al.'s observations of early diastolic dysfunction in SCD patients due to chronic anemia-related volume overload and vascular injury . Additionally, while most participants demonstrated normal systolic function, a few presented with mild systolic dysfunction, indicating that early ventricular strain could be present even in the absence of overt symptoms. The FICAT staging of avascular necrosis (AVN) showed that most participants had no AVN, with only a few in advanced stages, suggesting that musculoskeletal complications might not be prominent until later stages of disease progression. This finding is in line with **Dass** et al.'s work, which indicates a lower prevalence of AVN in milder cases of SCD .

Correlation of FICAT Staging with Hospitalizations and Hematological Parameters

FICAT staging correlated significantly with hospitalization frequency, as participants in advanced stages (2 and 3) were more likely to have frequent hospitalizations, potentially due to the increased pain and mobility issues associated with AVN. The higher hospitalization rates align with **Da Guarda** et al., who emphasize that musculoskeletal complications in SCD, such as AVN, can lead to increased healthcare utilization due to pain and functional impairment . Furthermore, Hemoglobin S (HbS) levels showed statistically significant variation across FICAT stages, with lower HbS levels in Stage 2 participants, which may reflect a compensatory increase in fetal hemoglobin (HbF) to mitigate sickling in some patients. Elevated HbF is known to protect against sickling, as observed by **Dass** et al. .

Renal and Cardiovascular Implications: Microalbuminuria and Diastolic Dysfunction

Microalbuminuria was present in a subset of participants, aligning with **Hulsizer** et al., who reported that renal complications in SCD could manifest early, often before significant renal function decline is detected . Additionally, the study's findings on diastolic dysfunction, predominantly mild, were consistent with **Nagel** et al.'s findings that diastolic dysfunction often precedes systolic dysfunction in SCD patients due to chronic volume overload . Although the association between hematological parameters and microalbuminuria was not statistically significant, the observed trends suggest that monitoring urinary albumin levels could help in early identification of renal dysfunction in SCD patients, as supported by **Chaturvedi** et al. .

D-Dimer as a Marker of Coagulation Activity

While D-dimer levels showed some trends toward elevation in groups with increased acute pain crises and hospitalizations, no statistically significant findings were noted. However, this trend does resonate with **Stein** et al., who found D-dimer to be a sensitive marker for coagulation activity in SCD patients, particularly during vaso-occlusive crises . This lack of statistical significance in the current study could again be related to sample size limitations. Nonetheless, D-dimer remains a valuable parameter to monitor, as elevated levels indicate a hypercoagulable state that may contribute to thrombotic complications.

Table 20 : Summary of Key findings.

Section	Findings	Statistical significance
SCD Prevalence and Demographics	Predominant demographic from Madhya Pradesh with high prevalence of SCD, especially in tribal areas (40%-55% sickle cell trait)	Not analyzed
Acute Pain Events and Hospitalizations	Half of participants reported low pain frequency; trends in elevated TLC and D-dimer with moderate pain frequency noted	Trends observed, no statistical significance
Correlation of Hematological Parameters with Hospitalization Frequency	No significant differences in hematological parameters by hospitalization frequency; elevated TLC and platelet counts in high hospitalization group observed	Not statistically significant; trends observed
Imaging Parameters: Cardiopulmonary and Musculoskeletal Implications	Diastolic dysfunction identified in some; mild systolic dysfunction noted, and low prevalence of AVN	Not statistically significant
Correlation of FICAT Staging with Hospitalizations and Hematological Parameters	Advanced FICAT stages correlated with higher hospitalizations and lower HbS levels, possibly due to increased HbF in advanced stages	Statistically significant correlation with hospitalization frequency
Renal and Cardiovascular Implications: Microalbuminuria and Diastolic Dysfunction	Microalbuminuria present in some, diastolic dysfunction observed, no significant association with hematological parameters	Trends observed, no statistical significance
D-Dimer as a Marker of Coagulation Activity	Elevated D-dimer levels in groups with higher acute pain and hospitalizations; non-significant but suggests hypercoagulable state	No statistical significance; trends suggestive

Summary

This cross-sectional study conducted at AIIMS Bhopal aimed to evaluate the relationship between hematological parameters, acute pain crisis frequency, and chronic complications in adult patients with Sickle Cell Anemia (SCD), with an additional focus on the role of imaging findings. A total of 42 participants, predominantly male and from Central India, were included, with data collected through hematological analysis, imaging studies, and clinical assessments.

Key Findings

1. Hematological Correlations with Acute Crises: Lower hemoglobin (Hb) levels and increased total leukocyte count (TLC) were noted among participants with frequent pain crises. While not statistically significant, these trends suggest a potential link between anemia severity and crisis frequency. Elevated D-dimer levels and higher HbS concentrations were observed in participants with more frequent crises, indicating heightened inflammation and hypercoagulability, consistent with increased erythropoietic activity seen in higher reticulocyte counts.

2. Imaging and Chronic Complications: Imaging via 2D echocardiography showed that while most participants maintained normal systolic function, a subset exhibited mild-to-moderate dysfunction, with lower Hb levels correlating with reduced ejection fraction. The Ficat and Arlet classification for avascular necrosis (AVN) revealed that the majority were Stage 0, with those in advanced stages exhibiting increased healthcare utilization and transfusion needs. X-ray and MRI findings identified classic SCD-related bone complications, and nuclear imaging underscored the utility of bone scans for distinguishing between infarcts and osteomyelitis.

3. Correlation of Imaging and Hematological Data: There was a notable relationship between imaging findings and HbS levels, where participants with higher Ficat stages and advanced AVN presented with elevated HbS and lower HbF levels. This association underscores the potential role of fetal hemoglobin

(HbF) in mitigating severe bone complications and aligns with findings by Adekile et al. on HbF's protective effects.

Limitations

The study's primary limitations include its cross-sectional design, which limits the ability to establish causality between hematological parameters and long-term complications. Additionally, the relatively small sample size and regional focus may reduce the generalizability of findings. Imaging modalities such as bone scans were only feasible for a subset, limiting comprehensive imaging-based conclusions across the full sample. Finally, the study's reliance on patient self-reporting for crisis frequency may introduce recall bias, affecting the accuracy of crisis-related data.

Implications

These findings suggest the importance of regular monitoring of HbF levels, HbS concentration, and reticulocyte indices in SCD patients, particularly for those with frequent crises and more severe phenotypes. Given the association of low Hb and high HbS with cardiac and musculoskeletal complications, the study underscores the need for personalized treatment plans, potentially incorporating hydroxyurea and comprehensive health education to mitigate acute and chronic complications in SCD.

In conclusion, this study highlights critical correlations between hematological and imaging parameters and SCD severity. Future longitudinal studies are recommended to further clarify these associations and explore targeted therapeutic approaches to improve outcomes in SCD.

Conclusions

This study presents a detailed characterization of the hematological, cardiovascular, and musculoskeletal features of SCD in a central Indian cohort. While the sample size and regional focus limit the generalizability of these findings, the data underscore the multifaceted nature of SCD and its complications. Further research with larger, multi-centric cohorts is needed to elucidate the observed trends and statistically validate the potential correlations identified here. Prospective studies focusing on the role of hydroxyurea therapy and compliance, along with regular monitoring of hematological and biochemical markers, will be critical in advancing SCD management strategies in resource-limited settings.

Overall, the findings align well with the broader literature on SCD, supporting **Stein** et al., **Nagel** et al., and others who have highlighted the utility of integrating hematological, imaging, and biochemical markers for comprehensive SCD management. As SCD care evolves, continued research will be essential in refining and adapting interventions that cater to the specific needs of SCD patients in diverse geographic and socioeconomic contexts.

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Annexure

Annexure 1 : Data Collection Form

DATA COLLECTION FORM

1. **NAME**

2. **AGE**

3. **SEX**

4. **ADDRESS**

5. **CR NO**

6. **SOCIOECONOMIC STRATA**

APL/BPL

7. **WEIGHT**
8. **HEIGHT**

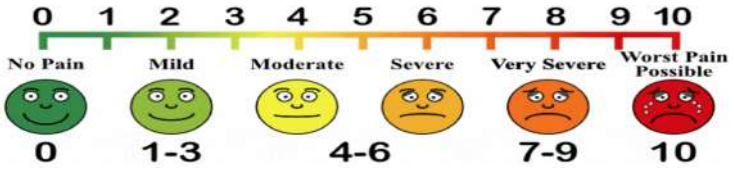
9. **BP**
10. **PULSE**

HISTORY

11. **ACUTE PAIN CRISIS -**

    a. FREQUENCY IN 12 MONTHS -

    b. Avg DURATION

    c. SEVERITY 

12. **HOSPITALISATION -**

    a. **TYPE**- 1' / 2' / 3' / PRIVATE CLINIC / PRIVATE HOSPITAL;

    b. **FREQUENCY IN 12 MONTHS** -

    c. **REASON** - PAIN / WEAKNESS / FEVER / OTHER

13. **BLOOD TRANSFUSION -**

    a. **FREQUENCY IN 12 MONTHS** -

    b. **NUMBER OF UNITS -**

14. **DRUGS-**

    HYDROXYUREA - Y/N;

    FOLIC ACID - Y/N;

    OTHERS-

15. **DOSAGE** - LOW DOSE/ HIGH DOSE

16. **COMPLIANCE -** Y/N

17. **HEALTH EDUCATION** - REGULAR / OCCASIONAL / NEVER

18. **LAB INVESTIGATIONS-**

CBC-
Hb
TLC
P%
L%
PLT
RETIC
HBS
HBA0
HBA2
HBF
D- DIMER
UREA
CREAT
URINE MICROALBUMIN

19. **IMAGING** - FICAT AND ARLET CLASSIFICATION

20. **CARDIAC** -

SYSTOLIC DYSFUNCTION
DIASTOLIC DYSFUNCTION

21. **RESP**-

PFT- RESTRICTIVE/OBSTRUCTIVE

22. **OTHERS**-

Annexure 2 : Participant informed consent form

प्रतिभागी सूचना सहमति प्रपत्र

[PARTICIPANT INFORMED CONSENT FORM (PICF)]

इस अध्ययन के लिए प्रतिभागी का पहचान नंबर/Participant Identification Number for this Study: ……

अनुसंधान का शीर्षक/Title of Project: Hematological and Imaging Parameters in prediction of adverse outcomes in Adult Sickle Cell Anemia

शोधकर्ता छात्र का नाम: डॉ आदर्श अरुण मढ़रिया फोन नं:.+91 76397536003

Name of Student Investigator :Dr ADARSH ARUN MADHARIA Tel No.(s) : +91 76397536003

शोध मार्गदर्शक का नाम / Name of Thesis Guide : DR VAIBHAV INGLE

मैंने दिनांक 19.01.2023 के सूचना पत्रक संस्करण II में दिए गए सभी तथ्यों को सावधानीपूर्वक पढ़ लिया है/ मुझे अपनी भाषा में स्पष्ट रूप से विस्तारपूर्वक समझा दिया गया है, और मैंने तथ्यों को पूर्णतः समझ लिया है। मैं पुष्टि करता/ करती हूँ, कि मुझे सवाल पूछने के अवसर प्रदान किए गए। The contents of the information sheet version II dated 19 January 2023 that was provided have been read carefully by me / explained in detail to me, in a language that I comprehend, and I have fully understood the contents. I confirm that I have had the opportunity to ask questions.

मुझे इस अध्ययन की प्रकृति एवं उद्देश्य और इसके संभावित जोखिमों / लाभों तथा अध्ययन की अनुमानित अवधि एवं अन्य प्रासंगिक जानकारी के बारे में विस्तारपूर्वक समझा दिया गया है। मुझे पता है कि इस अध्ययन में मेरी भागीदारी स्वैच्छिक है तथा मैं इस अध्ययन से किसी भी समय बिना कोई कारण बताए, अपना नाम वापस लेने के लिए स्वतंत्र हूं और नाम वापस लेने पर मेरी चिकित्सा देखभाल या कानूनी अधिकारों पर कोई प्रभाव नहीं पड़ेगा। The nature and purpose of the study and its potential risks/benefits and expected duration of the study, and other relevant details of the study have been explained to me in detail. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal right being affected.

मैं समझता/समझती हूं कि इस अनुसंधान में मेरी भागीदारी से मेरे बारे में एकत्र की गई जानकारी और मेरे चिकित्सीय दस्तावेजों के किसी भी भाग को एम्स के जिम्मेदार व्यक्तियों द्वारा ही देखा जाएगा। मैं इन व्यक्तियों को मेरे रिकॉर्ड प्राप्त करने की अनुमति प्रदान करता/ करती हूँ। I understand that the information collected about me from my participation in this research and sections of any of my medical notes may be looked at by responsible individuals from AIIMS. I give permission for this individuals to have access to my records.

यदि प्रतिभागी बच्चा है तो उसकी मौखिक या लिखित सहमति प्राप्त की गई है। If participants is child, his/her verbal of written assent has been obtained.

मैं उक्त अध्ययन में भाग लेने के लिए सहमत हूं/I agree to take part in the above study.

_____

(प्रतिभागी के हस्ताक्षर / बांए अंगूठे का निशान) (Signature/Left Thumb Impression)

दिनांक/Date……स्थान/Place ……

प्रतिभागी का नाम/Name of the participant……

पिता/पति/पत्नी का नाम /Son/Daughter/Spouse of……

डाक का पूरा पता /Complete Postal Address ……

अगर अशिक्षित हों / If illiterate.

सूचना पत्र को प्रतिभागी को पढ़ा और समझाया गया है और प्रश्न पूछने का अवसर दिया गया है। मैं पुष्टि करता हूं कि व्यक्ति ने स्वतंत्र रूप से अपनी सहमति दी है ।

The information sheet has been read and explained to the participant and given the opportunity to ask questions. I confirm that the individual has given consent freely.

कानूनी रूप से स्वीकार्य प्रतिनिधि या निष्पक्ष गवाह के हस्ताक्षर

Signature of Legally acceptable representative or impartial witness.

दिनांक/Date

एलएआर या निष्पक्ष गवाह का नाम / Name of LAR or impartial witness .

पता / Address :-

प्रमाणित किया जाता है कि उक्त सहमति मेरी उपस्थिति में प्राप्त की गई ।

This is to certify that the above consent has been obtained in my presence.

_____

छात्र शोधकर्ता के हस्ताक्षर दिनांक……स्थान……

Signature of the student Investigator Date …… Place ……..

Annexure 3 : Participant Information Sheet (Hindi)

प्रतिभागी सूचना पत्र

अध्ययन का शीर्षक:Hematological and Imaging Parameters in prediction of adverse outcomes in Adult Sickle Cell Anemia

अध्ययन जांचकर्ता

छात्र (नाम, विभाग) **डॉ आदर्श अरुण मढ़रिया**

मार्गदर्शक (गाइड) (नाम, विभाग) Dr Vaibhav Ingle, General Medicine

सह मार्गदर्शक/कों (नाम, विभाग) Dr Rajnish Joshi, General Medicine

Dr E Jayashankar, Pathology and Laboratory Medicine

Dr Sachin Bansal, Medical Oncology and Hematology

Dr Jitendra Sharma, Radiology

Dr Suruchi Jain, Nuclear Medicine

परिचयः

हम एम्स, भोपाल में Hematological and Imaging Parameters in prediction of adverse outcomes in Adult Sickle Cell Anemia विषय पर अनुसंधान, अध्ययन कर रहे हैं । यदि आप स्वेच्छा से भाग लेने के लिए सहमत है तो, हम आपसे यह सहमति प्रपत्र हस्ताक्षर करने के लिए आग्रह करेंगे।

अध्ययन का उद्देश्यः

हमारे अध्ययन का उद्देश्य है सिकल सेल एनीमिया के रोगियों में रक्त और रेडियोलॉजिकल इमेजिंग के द्वारा बीमारी से होने वाले दुष्परिणाम का समय से पहले पता लगाना इससे हमें दुष्परिणाम की खोज में सहायता मिलेगी।

भाग लेना -

A. **इसमें क्या शामिल है ?**

इस अनुशंधान का हिस्से बनने पर आपसे आपके बीमारी के बारे में जानकारी पूछी जाएगी , आपका रक्त व पेशाब जांच एकत्रित किये जायेंगे | उसके अलावा कुछ रेडियोलॉजिकल जाचे जैसे X ray , सी टी स्कैन व बोन स्कैन भी कराया जा सकता है |

B. **क्या मुझे भाग लेना चाहिए ?**

इस अनुसंधान में भाग लेना पूर्णतः स्वैच्छिक है । भाग नहीं लेने का निर्णय या किसी भी समय अपना नाम वापस लेने से, किसी भी रूप में आपके अधिकारों या आपकी चिकित्सा देखभाल पर कोई विपरीत प्रभाव नहीं पड़ेगा ।

C. **यदि मैं इसमें शामिल होता हूं तो मुझसे क्या अपेक्षित हैं ?**

यदि आप इसमें शामिल होते हैं तो , हम सिकल सेल एनीमिया के बारे में सूचना एकत्रित करेंगे |

D. **अध्ययन में मेरी भागीदारी कब तक चलेगी ?**

अध्ययन में आपकी भागीदारी 18 महीने या अध्ययन ख़तम होते तक होगी |

E. **क्या मुझे इस अध्ययन के लिए अलग से आना पड़ेगा या अतिरिक्त जांच जरूरी होगी ।**

मरीज को अतिरिक्त जांच की आवश्यकता हो सकती है जैसे सीरम एनटी प्रोबीएनपी, एक्स रे, एचआरसीटी थोरैक्स और बोन स्कैन । यह सारे उपर्लिखित जाचे सामान्य तौर पर सिकल सेल एनीमिया से होने वाले लम्बे समय के दुष्परिणाम को जल्द पहचानने के लिए किये जाते है।

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F. **इसमें शामिल होने से संभावित लाभ क्या है ?**

प्रत्यक्ष अथवा अप्रत्यक्ष लाभ जैसे, भाग लेने से **सिकल सेल एनीमिया** के बारे में ज्ञान की वृद्धि होगी। इसके परिणामस्वरूप आपके / आप जैसे मरीजों के प्रबंधन/परिणामों को बेहतर बनाया जा सकता है।

G. **इसमें भाग लेने से संभावित हानि एवं जोखिम ?**

इस अध्ययन के कारण किसी बड़ी हानि या जोखिम की पूर्व संभावना नहीं है। एकत्र की गई सूचना एवं आंकड़ों एवं इनकी एक्सेस को सुरक्षित रखने की सभी सावधानियों के बाद भी इन सूचनाओं की निजता अथवा गोपनीयता को क्षति पहुँचने का जोखिम रहेगा ।

H. **मेरे बारे में एकत्र की गई सूचना का क्या किया जाएगा ?**

एकत्र की गई सभी सूचना पूर्णतः गोपनीय रखी जाएगी तथा अध्ययन से प्राप्त निष्कर्षों को अन्य किसी से साझा नहीं किया जाएगा । इन्हें ग्रुप आंकड़े के रूप मे रिपोर्ट किया जाएगा और किसी भी तरह से आपकी पहचान उजागर नहीं होगी ।

I. **इस अध्ययन मे भाग लेने के लिए मेरे विचार बदलने पर क्या होगा ?**

अध्ययन के दौरान किसी भी समय बिना किसी क्षति या दंड के आपको अपने विचार बदलने का पूरा अधिकार है ।

J. **इस संबंध मे अधिक जानकारी के लिए या बाद मे मेरे मन मे कोई प्रश्न या शंका होने पर मुझ किनसे संपर्क करना होगा ?**

यदि आप कोई सूचना चाहते हैं या आपका कोई प्रश्न है या शंका है तो आप इन नंबरों पर संपर्क कर सकते हैं: +91 7697536003, +91 9340945522

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Annexure 4 : Participant Information Sheet (English)

Participant Information Sheet

A. STUDY TITLE: Hematological and Imaging Parameters in prediction of adverse outcomes in Adult Sickle Cell Anemia

B. STUDY INVESTIGATORS:

a. **Student:** Dr Adarsh Arun Madharia, Dept of General Medicine
b. **Guide** : Dr Vaibhav Ingle, Department of General Medicine
c. **Co- Guide/s**:
Dr RajnishJoshi, Department of General Medicine
Dr E Jayashankar, Dept of Pathology and Laboratory Medicine
Dr Sachin Bansal, Dept of MedicalOncologyandHematology
Dr Jitendra Sharma, Dept of Radiodiagnosis
Dr Suruchi Jain, Dept of Nuclear Medicine

C. Introduction:

We are conducting a research study to determine the hematological and imaging parameters in prediction of adverse outcomes in adult sickle cell disease in AIIMS Bhopal. We would like to study for the hematological and imaging parameters. If you agree to take part voluntarily, we will ask you to sign a consent form.

D. Purpose of the study:

The aim of our study is to determine the performance of nuclear baseline hematological and imaging parameters in predicting vaso-occlusive Sickle cell crises (bone infarcts/ avascular necrosis/stroke/splenic infarcts) and chronic complications. This will help us in predicting the adverse outcomes of adult sickle cell anemia

E. Taking part – what it involves:

a. Do I have to take part?

Participation in this research is entirely voluntary. A decision to not take part, or to withdraw at any time, will not affect your rights or medical care in any way.

b. What is expected of me if I take part?

If you participate, we will collect information about sickle cell anemia and collect samples

for hematological and biochemical studies. You may also be exposed to some radiological examinations such as Xray , MRI Hip and/or Bone Scan.

c. How long will my part in the study last?

Your participation in the study would last for 18 months or till the end of study duration, whichever comes first.

d. Do I have to come for extra visits or undergo extra investigations for the study?

You would be required to undergo blood tests and radiological tests like Xray, CT Scan, and/or bone scan. These above mentioned tests are routine for early detection of long term complications in adults with sickle cell anemia

e. What are the possible benefits in taking part?

Taking part will help gain knowledge about sickle cell anemia which may result in improvement of management / outcomes for you / patients like yourself.

f. What are the possible disadvantages and risks of taking part?

There are no major foreseeable disadvantages or risks because of the study. There may be risk of loss of privacy and confidentiality of information collected even though all possible measures will be taken to ensure safe data storage and access.

g. What happens to the information collected from me?

All information collected will be kept strictly confidential and not shared with anyone. Results from the study will be reported as group data and will not identify you in any way.

h. What if I change my mind regarding participation in the study?

You are entitled to change your mind and withdraw at any time during the study without any disadvantage or penalty.

i. Whom do I contact for more information or if I have further questions or concerns?

If you want any information or have questions or concerns, you can contact:
+91-7697536003, +91-9340945522

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Dr. Shashank Purwar
Member Secretary,
Institute Ethics Committee – Student

Master Chart

S No	AGE	SEX	ADDRESS	SOCIOECONOMIC STATUS	WEIGHT	HEIGHT	HEIGHT (m)	BMI	SBP	DBP	PULSE
1	19	M	HOSHANGABAD, MP	APL	39	176.0	1.76	12.59	122	84	78
2	46	M	BHOPAL, MP	BPL	45	168.0	1.68	15.94	100	60	68
3	20	M	NARSIMHAPUR, MP	BPL	49	170.0	1.7	16.96	126	78	72
4	25	M	GONDIA, MH	APL	53	166.0	1.66	19.23	125	74	95
5	20	F	BETUL, MP	BPL	35	155.0	1.55	14.57	102	70	94
6	19	M	BHOPAL, MP	APL	52	172.0	1.72	17.58	114	66	110
7	21	M	BHOPAL, MP	APL	62	166.0	1.66	22.50	112	68	98
8	41	F	BHOPAL, MP	APL	68	166.0	1.66	24.68	118	70	78
9	23	M	RAISEN, MP	BPL	47	159.0	1.59	18.59	126	82	70
10	25	F	BHOPAL, MP	APL	38	152.0	1.52	16.45	116	78	76
11	18	M	BHOPAL, MP	APL	58	160.0	1.6	22.66	112	68	88
12	35	M	CHHINDWARA, MP	APL	46	168.0	1.68	16.30	132	86	94
13	20	M	RAISEN, MP	APL	46	174.0	1.74	15.19	112	64	89
14	34	M	BHOPAL, MP	APL	57	172.0	1.72	19.27	116	76	76
15	38	F	BHOPAL, MP	APL	47	156.0	1.56	19.31	103	62	65
16	49	M	HOSHANGABAD, MP	BPL	52	164.0	1.64	19.33	98	58	78
17	18	F	SEHORE, MP	BPL	44	162.0	1.62	16.77	96	58	70
18	42	F	NARSIMHAPUR, MP	APL	64	170.0	1.7	22.15	118	78	88
19	20	M	HOSHANGABAD, MP	BPL	48	168.0	1.68	17.01	112	64	78
20	27	M	HOSHANGABAD, MP	BPL	52	166.0	1.66	18.87	120	78	90
21	19	M	BETUL, MP	BPL	45	165.0	1.65	16.53	116	76	108
22	40	F	BHOPAL, MP	APL	74	165.0	1.65	27.18	148	90	88
23	27	F	SAGAR, MP	APL	42	162.0	1.62	16.00	118	60	72
24	20	F	BHOPAL, MP	BPL	42	155.0	1.55	17.48	112	70	86
25	18	M	HOSHANGABAD, MP	APL	46	160.0	1.6	17.97	120	70	96
26	33	M	BHOPAL, MP	APL	68	162.0	1.62	25.91	124	82	84
27	23	F	BHOPAL, MP	APL	46	164.0	1.64	17.10	118	70	98
28	27	F	BHOPAL, MP	APL	50	163.0	1.63	18.82	124	78	86
29	40	M	HOSHANGABAD, MP	APL	58	166.0	1.66	21.05	102	64	72
30	20	F	BETUL, MP	APL	36	164.0	1.64	13.38	132	64	75
31	34	M	BHOPAL, MP	APL	56	168.0	1.68	19.84	110	76	68
32	24	M	VIDISHA, MP	BPL	47	170.0	1.7	16.26	110	66	83
33	28	M	BHOPAL, MP	BPL	46	172.0	1.72	15.55	118	76	96
34	37	F	BETUL, MP	BPL	40	166.0	1.66	14.52	124	72	88
35	25	M	HOSHANGABAD, MP	APL	55	170.0	1.7	19.03	122	78	88
36	19	M	BHOPAL, MP	APL	50	172.0	1.72	16.90	120	68	70
37	23	M	GONDIA, MH	APL	52	175.0	1.75	16.98	118	78	80
38	39	M	BELAGHAT, MP	APL	45	168.0	1.68	15.94	104	68	74
39	25	M	HOSHANGABAD, MP	BPL	45	166.0	1.66	16.33	105	60	110
40	20	F	SEHORE, MP	BPL	35	160.0	1.6	13.67	100	68	84
41	24	M	HOSHANGABAD, MP	APL	48	170.0	1.7	16.61	112	76	78
42	23	M	BETUL, MP	APL	44	172.0	1.72	14.87	124	82	68

S No	AGE	AIN FREQUENCY(in 12 monthst	PAIN FREQUENCY (in 12 month	PAIN DURATION (days)	Cat Pain Duration (days)	PAIN SEVERITY
1	19	15	VERY HIGH(>12)	2	SHORT(0-3)	4 to 6
2	46	0	LOW(0-2)	0	SHORT(0-3)	0
3	20	6	MODERATE(3-6)	4	MODERATE(4-6)	4 to 6
4	25	2	LOW(0-2)	4	MODERATE(4-6)	7 to 9
5	20	3	MODERATE(3-6)	7	LONG(7-10)	7 to 9
6	19	12	HIGH(7-12)	4	MODERATE(4-6)	7 to 9
7	21	8	HIGH(7-12)	5	MODERATE(4-6)	4 to 6
8	41	3	MODERATE(3-6)	3	SHORT(0-3)	7 to 9
9	23	0	LOW(0-2)	0	SHORT(0-3)	0
10	25	0	LOW(0-2)	0	SHORT(0-3)	0
11	18	1	LOW(0-2)	5	MODERATE(4-6)	7 to 9
12	35	6	MODERATE(3-6)	4	MODERATE(4-6)	7 to 9
13	20	6	MODERATE(3-6)	2	SHORT(0-3)	7 to 9
14	34	4	MODERATE(3-6)	8	LONG(7-10)	7 to 9
15	38	15	VERY HIGH(>12)	6	MODERATE(4-6)	7 to 9
16	49	2	LOW(0-2)	4	MODERATE(4-6)	1 to 3
17	18	0	LOW(0-2)	0	SHORT(0-3)	0
18	42	2	LOW(0-2)	4	MODERATE(4-6)	7 to 9
19	20	2	LOW(0-2)	7	LONG(7-10)	7 to 9
20	27	2	LOW(0-2)	7	LONG(7-10)	4 to 6
21	19	3	MODERATE(3-6)	3	SHORT(0-3)	7 to 9
22	40	4	MODERATE(3-6)	5	MODERATE(4-6)	7 to 9
23	27	8	HIGH(7-12)	10	LONG(7-10)	7 to 9
24	20	0	LOW(0-2)	0	SHORT(0-3)	0
25	18	3	LOW(0-2)	2	SHORT(0-3)	1 to 3
26	33	0	LOW(0-2)	0	SHORT(0-3)	0
27	23	4	MODERATE(3-6)	2	SHORT(0-3)	4 to 6
28	27	0	LOW(0-2)	0	SHORT(0-3)	0
29	40	0	LOW(0-2)	0	SHORT(0-3)	0
30	20	2	LOW(0-2)	15	VERY LONG(>10)	4 to 6
31	34	4	MODERATE(3-6)	7	MODERATE(4-6)	7 to 9
32	24	1	LOW(0-2)	4	MODERATE(4-6)	7 to 9
33	28	3	MODERATE(3-6)	4	MODERATE(4-6)	7 to 9
34	37	4	MODERATE(3-6)	3	SHORT(0-3)	4 to 6
35	25	0	LOW(0-2)	0	SHORT(0-3)	0
36	19	6	MODERATE(3-6)	4	MODERATE(4-6)	4 to 6
37	23	4	MODERATE(3-6)	3	SHORT(0-3)	7 to 9
38	39	8	HIGH(7-12)	4	MODERATE(4-6)	7 to 9
39	25	1	LOW(0-2)	2	SHORT(0-3)	1 to 3
40	20	2	LOW(0-2)	10	LONG(7-10)	7 to 9
41	24	4	MODERATE(3-6)	7	LONG(7-10)	4 to 6
42	23	12	HIGH(7-12)	8	LONG(7-10)	4 to 6

S.No	AGE	HEALTH CARE TYPE(First and most frequent contact)	Frequency of health care facility visit in 12 months
1	19	Private clinic	6
2	46	Tertiary	1
3	20	Tertiary	2
4	25	Tertiary	2
5	20	Tertiary	3
6	19	Tertiary	4
7	21	Tertiary	6
8	41	Private clinic	3
9	23	Primary	2
10	25	Tertiary	1
11	18	Tertiary	1
12	35	Tertiary	2
13	20	Private clinic	4
14	34	Private hospital	3
15	38	Secondary	20
16	49	Primary	2
17	18	Tertiary	1
18	42	Private hospital	2
19	20	Tertiary	3
20	27	Tertiary	1
21	19	Tertiary	4
22	40	Private hospital	4
23	27	Private clinic	12
24	20	Tertiary	2
25	18	Private clinic	1
26	33	Private clinic	1
27	23	Tertiary	2
28	27	Tertiary	0
29	40	Private hospital	2
30	20	Tertiary	2
31	34	Private clinic	4
32	24	Tertiary	6
33	28	Tertiary	4
34	37	Secondary	2
35	25	Private clinic	4
36	19	Private clinic	8
37	23	Tertiary	2
38	39	Tertiary	4
39	25	Private clinic	0
40	20	Private clinic	2
41	24	Private clinic	2
42	23	Tertiary	6

S No	AGE	Cat Frequency of Heathcare visit(12 months)	REASON FOR VISIT	Blood Transfusion frequency in last 12 months
1	19	HIGH(3-10)	fever	0
2	46	LOW(0-2)	weakness	0
3	20	LOW(0-2)	pain	0
4	25	LOW(0-2)	pain	0
5	20	HIGH(3-10)	pain	1
6	19	HIGH(3-10)	pain	0
7	21	HIGH(3-10)	others	4
8	41	HIGH(3-10)	pain	0
9	23	LOW(0-2)	weakness	0
10	25	LOW(0-2)	weakness	1
11	18	LOW(0-2)	pain	0
12	35	LOW(0-2)	pain	0
13	20	HIGH(3-10)	pain	0
14	34	HIGH(3-10)	pain	8
15	38	VERY HIGH(>10)	pain	0
16	49	LOW(0-2)	weakness	4
17	18	LOW(0-2)	fever	0
18	42	LOW(0-2)	pain	0
19	20	HIGH(3-10)	pain	1
20	27	LOW(0-2)	pain	0
21	19	HIGH(3-10)	pain	0
22	40	HIGH(3-10)	pain	3
23	27	VERY HIGH(>10)	pain	0
24	20	LOW(0-2)	others	0
25	18	LOW(0-2)	pain	0
26	33	LOW(0-2)	weakness	2
27	23	LOW(0-2)	weakness	1
28	27	LOW(0-2)	weakness	0
29	40	LOW(0-2)	weakness	2
30	20	LOW(0-2)	pain	0
31	34	HIGH(3-10)	pain	4
32	24	HIGH(3-10)	others	1
33	28	HIGH(3-10)	pain	2
34	37	LOW(0-2)	weakness	2
35	25	HIGH(3-10)	others	0
36	19	HIGH(3-10)	pain	0
37	23	LOW(0-2)	pain	0
38	39	HIGH(3-10)	weakness	2
39	25	LOW(0-2)	fever	0
40	20	LOW(0-2)	pain	0
41	24	LOW(0-2)	pain	0
42	23	HIGH(3-10)	pain	2

S No	AGE	Units of PRBC transfused in the last 12 months	HYDROXYUREA	FA	Dose	COMPLIANCE	HEALTH EDUCATION
1	19	0	Y (HU)	Y(FA)	Low Dose	y	occasional
2	46	0	Y (HU)	Y(FA)	High Dose	y	regular
3	20	0	Y (HU)	Y(FA)	Low Dose	y	occasional
4	25	0	Y (HU)	Y(FA)	High Dose	y	regular
5	20	2	Y (HU)	Y(FA)	High Dose	y	never
6	19	0	Y (HU)	Y(FA)	Low Dose	n	regular
7	21	6	Y (HU)	Y(FA)	High Dose	y	regular
8	41	0	Y (HU)	Y(FA)	Low Dose	y	regular
9	23	0	Y (HU)	N (FA)	Low Dose	n	occasional
10	25	4	Y (HU)	Y(FA)	High Dose	n	occasional
11	18	0	Y (HU)	Y(FA)	Low Dose	n	regular
12	35	0	Y (HU)	Y(FA)	Low Dose	y	occasional
13	20	0	N (HU)	Y(FA)	Low Dose	n	occasional
14	34	5	Y (HU)	N (FA)	Low Dose	n	occasional
15	38	0	Y (HU)	Y(FA)	Low Dose	n	regular
16	49	6	Y (HU)	Y(FA)	High Dose	y	regular
17	18	0	Y (HU)	Y(FA)	High Dose	y	regular
18	42	0	Y (HU)	Y(FA)	High Dose	y	occasional
19	20	2	Y (HU)	Y(FA)	High Dose	y	regular
20	27	0	Y (HU)	Y(FA)	High Dose	n	regular
21	19	0	Y (HU)	Y(FA)	High Dose	n	occasional
22	40	3	Y (HU)	Y(FA)	High Dose	n	occasional
23	27	0	Y (HU)	Y(FA)	High Dose	y	occasional
24	20	0	Y (HU)	Y(FA)	Low Dose	n	never
25	18	0	Y (HU)	Y(FA)	Low Dose	y	occasional
26	33	12	N (HU)	N (FA)	Low Dose	n	never
27	23	2	Y (HU)	Y(FA)	Low Dose	n	regular
28	27	0	Y (HU)	Y(FA)	Low Dose	n	regular
29	40	5	N (HU)	N (FA)	Low Dose	n	never
30	20	0	Y (HU)	Y(FA)	High Dose	y	occasional
31	34	6	Y (HU)	Y(FA)	Low Dose	y	occasional
32	24	2	Y (HU)	N (FA)	High Dose	y	regular
33	28	6	Y (HU)	Y(FA)	Low Dose	n	regular
34	37	4	Y (HU)	Y(FA)	Low Dose	y	regular
35	25	0	N (HU)	N (FA)	Low Dose	n	occasional
36	19	0	Y (HU)	Y(FA)	Low Dose	n	occasional
37	23	0	Y (HU)	Y(FA)	Low Dose	n	regular
38	39	3	Y (HU)	Y(FA)	High Dose	y	regular
39	25	0	Y (HU)	Y(FA)	Low Dose	n	never
40	20	0	N (HU)	N (FA)	Low Dose	n	occasional
41	24	0	N (HU)	Y(FA)	Low Dose	n	occasional
42	23	4	Y (HU)	Y(FA)	High Dose	y	regular

S No	AGE	Hb	TLC	PLT	P%	L%	RETIC	HBS	HBA0	HBA2	HBF	LC Interpretati
1	19	7.6	9.02	321	38.7	50.9	6.52	83.7	2.4	2.8	9.3	SS
2	46	10.5	8.2	393	44.1	43.8	7.32	67	9.3	5.1	15.5	SB+
3	20	10.1	20.8	392	75.3	10.7	8.92	82.3	3.4	3	7.3	SS
4	25	9.9	17.69	398	66.3	26.4	5.96	75.1	2	4.2	18.7	SB+
5	20	7	11.25	503	70	18.3	6.53	77	6	3.3	11	SB+
6	19	8.7	4.29	135	60.3	30.8	6.11	70.1	2.4	1.4	22.6	SS
7	21	7.5	8.07	171	68.1	23.9	3.93	62.5	3.2	1.4	26	SS
8	41	9.8	9.68	454	41.9	48.1	8.7	71.7	4.3	1.6	18.7	SS
9	23	11.4	7.11	341	40.4	41.4	10.5	64.5	4.8	4.1	20.8	SB+
10	25	3.6	18.5	135	80	11.25	18.5	70.1	6.7	5.2	14.7	SB+
11	18	8.8	7.19	328	29	55	6.41	74.1	4.9	3.5	13.1	SB+
12	35	6.4	11.57	730	51.4	31.5	11.98	83.8	4.2	2.4	7.7	SS
13	20	8	17.27	278	56.7	31.4	8.15	65.9	4.5	1.1	24.6	SS
14	34	6.9	23.51	378	71.8	24.9	6.4	69.9	8.2	0.6	21.3	SB+
15	38	7.2	14.06	555	56	35.2	8.06	71.1	9.2	5	10.3	SB+
16	49	5.1	7.67	154	58.1	29.9	3.29	78.9	6.9	1.8	12.4	SB+
17	18	9.2	3.15	177	46.7	44.8	2.1	46.6	3.9	0.1	40.2	SS
18	42	8.3	22.91	420	71.3	16.5	7.49	82	4.4	3.2	7	SS
19	20	11	14.28	549	75.9	15.5	3.04	62	3.5	1.6	25.1	SS
20	27	8.6	20.42	241	72.2	15.8	20	72.7	4.1	5.3	14.2	SB+
21	19	10.3	16.5	207	48.9	45	11.12	67.8	4.7	1.7	22.2	SS
22	40	5.3	42.35	99	15.6	68.9	12.27	68.8	9.4	3.2	18.6	SB+
23	27	8.9	10.53	352	47.8	41.1	10.85	70.8	5.2	2.2	17.5	SB+
24	20	10.1	5.39	156	49.9	43.6	5.39	48.9	40.2	3.4	7.5	SB0
25	18	10.9	5	113	63	29.6	8.4	57	4.6	0.2	47	SS
26	33	5.9	4.33	237	50	39.3	2.63	42.4	53.2	3.6	0.8	AS
27	23	6.7	5.49	92	42.7	49.6	8.2	75.2	5.2	4.5	15.1	SB+
28	27	7.9	6.7	432	50.4	46	2.2	75.5	6.6	3.9	14	SB+
29	40	6	8.17	206	41	32	8.14	47.3	28.8	2.5	14.1	SB0
30	20	10.2	12.89	211	66.1	29.6	9.12	65.9	4.5	1.1	24.5	SS
31	34	7.8	24.57	420	81.7	12.3	3.4	78.6	3.6	2.2	15.6	SS
32	24	10.2	6.03	236	62	26.4	6	74.4	5.1	3	17.5	SB+
33	28	8.1	10.86	113	69	23	9.8	46.3	34.8	3	8.6	SB0
34	37	5	8.25	485	49.6	28.5	15.13	72	2.7	2.2	21	SS
35	25	9	7.42	245	76.2	19.1	12.4	82	3.4	2.8	11.8	SS
36	19	10.6	16.5	389	68.2	21.7	8.8	79.2	2.7	6	9.6	SB+
37	23	13	5.07	183	54.8	37.5	3.91	62.2	2.5	1.3	28.4	SS
38	39	6.2	3.48	110	52.9	38.4	0.41	40	11.6	3.9	38.8	SB+
39	25	7.8	14.97	103	69.2	15.8	5.96	64.7	6.5	2.4	23.5	SS
40	20	7.9	11.58	241	67.9	24.1	7.34	69.2	3.2	1.2	23.6	SS
41	24	7.1	16.71	417	83.4	8.6	9.8	82.7	2	3.4	10.7	SS
42	23	9.1	9.43	106	58.1	23.5	8.8	33.4	57	2.8	6.8	AS

S No	AGE	bF Interpretatic	D DIMER	UREA	CREAT	MICROALBUMINUR	UACR	BNP	FICAT
1	19	Low(<20)	2.2	12.08	0.56	11	N	10	0
2	46	Low(<20)	1.17	26.25	0.78	324	MACRO	1024	0
3	20	Low(<20)	2.1	14.36	0.6	5.5	N	10	0
4	25	Low(<20)	2.2	12.36	0.63	1.8	N	10	0
5	20	Low(<20)	2.47	18.96	0.64	2.1	N	10	0
6	19	High(>20)	1.22	21.43	0.61	2.2	N	10	3
7	21	High(>20)	0.55	20.57	0.71	2.5	N	10	0
8	41	Low(<20)	0.59	8.62	0.45	21	N	10	3
9	23	High(>20)	2.88	20.18	0.66	9.02	N	10	2
10	25	Low(<20)	0.6	19.57	0.6	24	N	10	0
11	18	Low(<20)	0.8	12.54	0.58	11	N	10	0
12	35	Low(<20)	2.9	24.21	0.94	1.4	N	10	0
13	20	High(>20)	1.28	20.63	0.56	1.66	N	10	0
14	34	High(>20)	2.95	7.03	0.48	0.82	N	10	0
15	38	Low(<20)	2	9.48	0.49	16	N	10	0
16	49	Low(<20)	2.98	97.53	1.64	5373	MACRO	255	0
17	18	High(>20)	2.6	18.57	0.59	14.8	N	10	0
18	42	Low(<20)	2.8	16.81	0.67	2.4	N	10	0
19	20	High(>20)	1.6	28.14	0.7	2.54	N	10	3
20	27	Low(<20)	2.3	20.44	0.75	0.8	N	10	0
21	19	High(>20)	2.59	36.59	0.54	6.88	N	10	2
22	40	Low(<20)	2.9	275.71	5.61	128.71	MICRO	368	0
23	27	Low(<20)	0.34	10.78	0.57	1.8	N	10	3
24	20	Low(<20)	2.97	22.5	0.72	8.72	N	10	0
25	18	High(>20)	1.84	12.93	0.66	33	MICRO	10	0
26	33	Low(<20)	0.48	24.66	0.45	28.56	N	10	0
27	23	Low(<20)	2.78	15.16	0.58	1.6	N	10	0
28	27	Low(<20)	2.88	21.8	0.64	0.8	N	10	0
29	40	Low(<20)	1.9	102	1.17	1442	MACRO	1595	0
30	20	High(>20)	1.99	20.63	0.56	1.66	N	10	0
31	34	Low(<20)	1.34	55.33	0.75	373.55	MACRO	10	3
32	24	Low(<20)	2.1	11.64	0.63	28.2	N	26	0
33	28	Low(<20)	2.41	29.09	0.97	18.2	N	10	2
34	37	High(>20)	0.61	22.8	0.57	48	MICRO	88	0
35	25	Low(<20)	2.88	34.8	0.76	14.8	N	10	0
36	19	Low(<20)	1.2	28.8	0.89	21.46	N	10	0
37	23	High(>20)	1.4	13.93	0.83	2.2	N	10	0
38	39	High(>20)	0.05	10.44	0.73	11.89	N	10	2
39	25	High(>20)	0.51	11.75	0.54	11.92	N	10	0
40	20	High(>20)	0.97	29.15	0.5	18.1	N	10	4
41	24	Low(<20)	22.2	21.85	0.59	25.6	N	10	0
42	23	Low(<20)	1.62	11.63	0.66	1.91	N	10	2

S.No	AGE	SYSTOLIC DYSFUNCTION	DIASTOLIC DYSFUNCTION
1	19	Normal	Normal
2	46	Normal	DYS 1
3	20	Normal	Normal
4	25	Normal	Normal
5	20	Normal	Normal
6	19	Normal	Normal
7	21	Normal	Normal
8	41	Normal	Normal
9	23	Normal	DYS 1
10	25	Normal	Normal
11	18	Normal	Normal
12	35	Normal	Normal
13	20	Normal	Normal
14	34	Normal	Normal
15	38	Normal	Normal
16	49	SYS 2	DYS 1
17	18	Normal	Normal
18	42	Normal	Normal
19	20	Normal	Normal
20	27	Normal	Normal
21	19	Normal	Normal
22	40	SYS 1	DYS 1
23	27	Normal	Normal
24	20	Normal	Normal
25	18	Normal	Normal
26	33	Normal	Normal
27	23	Normal	DYS 1
28	27	Normal	Normal
29	40	SYS 2	DYS 1
30	20	Normal	Normal
31	34	Normal	Normal
32	24	Normal	Normal
33	28	Normal	Normal
34	37	Normal	Normal
35	25	Normal	Normal
36	19	Normal	Normal
37	23	Normal	Normal
38	39	Normal	Normal
39	25	Normal	Normal
40	20	Normal	Normal
41	24	Normal	Normal
42	23	Normal	Normal